Rhodamine 6GX; C.I. Basic Red 1, Monohydrochloride; Elcozine Rhodamine 6GDN; Eljon Pink Toner; Fanal Pink GFK; Fanal Red 25532; Flexo Red 482; Heliostable Brilliant Pink B extra; Mitsui Rhodamine 6GCP; Nyco Liquid Red GF; Rhodamine 69DN Extra; Rhodamine F4G; Rhodamine F5G; Rhodamine F5G chloride; Rhodamine 6GB; Rhodamine 6GBN; Rhodamine 6GCP; Rhodamine 6GD; Rhodamine 4GD; Rhodamine GDN; Rhodamine 5GDN; Rhodamine 6 GDN; Rhodamine GDN Extra; Rhodamine 6GEx ethyl ester; Rhodamine 6G Extra; Rhodamine 6G Extra Base; Rhodamine 4GH; Rhodamine 6GH; Rhodamine 5GL; Rhodamine 6G lake; Rhodamine 6GX; Rhodamine J; Rhodamine 6JH; Rhodamine 7JH; Rhodamine Lake Red 6G; Rhodamine Y 20-7425; Rhodamine Zh; Rhodamine 6ZH-DN; Silosuper Pink B; Valley Fast Red 1308

Report Date: September 1989

TR-365 Toxicology and Carcinogenesis Studies of Pentaerythritol Tetranitrate (CAS No. 78-11-5) with 80% D-Lactose Monohydrate (PETN, NF) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Pentaerythritol tetranitrate (PETN, NF) is a drug used to prevent angina pectoris. PETN without a lactose stabilizer is used as an explosive. Toxicology and carcinogenesis studies were conducted by administering PETN, NF, to groups of F344/N rats and B6C3F$_1$ mice of each sex once by gavage or in feed for 14 days, 13 or 14 weeks, or 2 years. The PETN component was greater than 99% pure. Genetic toxicology studies were conducted with *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day and Thirteen-Week Studies: All rats and mice lived to the end of the 14-day studies (dietary concentrations up to 50,000 ppm). Final mean body weights of dosed and control rats were comparable. The final mean body weight of female mice that received 50,000 ppm was 13% lower than that of controls. No clinical signs or toxic lesions were attributed to PETN, NF, administration.

All rats and mice lived to the end of the 13-week (mice) and 14-week (rats) studies (dietary concentrations up to 50,000 ppm). Final mean body weights of dosed and control rats and mice were similar, although weight gains of female rats at 25,000 and 50,000 ppm were less than that of controls. The nitrite level in urine of rats and methemoglobin levels in whole blood of rats and mice were not affected by administration of PETN, NF. An adenoma of the Zymbal gland was seen in a female rat that received 50,000 ppm. A hepatocellular adenoma was seen in a female mouse that received 50,000 ppm.

Based on these results and the NTP convention of limiting concentrations in 2-year feed studies to 5% of the diet, the 2-year studies were conducted by administering 0, 25,000 or 50,000 ppm PETN, NF, in feed for 104 weeks to groups of 50 male rats and for 103 weeks to groups of 49 or 50 mice of each sex. Groups of 50 female rats were given feed containing 0, 6,200, or 12,500 ppm PETN, NF, for 104 weeks.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 2%-9% lower than those of controls throughout the study; body weights of all groups of female rats were similar. No significant differences in survival were observed between any groups of rats of either sex (male: control, 23/50; low dose, 29/50; high dose, 29/50; female: 33/50; 33/50; 31/50). Mean body weights of dosed and control mice were similar. The survival of both groups of dosed male mice was significantly greater than that of the controls (26/49; 38/50; 38/50). No significant differences in survival were observed between any groups of female mice (38/50; 30/50; 38/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: No nonneoplastic lesions were attributed to PETN, NF, administration in rats or mice. Neoplasms of the Zymbal gland occurred in dosed male (control, 0/49; low dose, 3/45; high dose, 2/41) and dosed female (0/36; 1/37; 3/35) rats. The historical incidence of these neoplasms is 1% ± 2% in untreated males and 0.6% ± 1% in females.

At no site was a significantly increased incidence of neoplasms observed in dosed male or female mice.

Genetic Toxicology: PETN, NF, was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation (S9). When tested for cytogenetic effects in cultured CHO cells, PETN, NF, induced sister chromatid exchanges (SCEs) in the presence and absence of metabolic activation; no induction of chromosomal aberrations was observed in CHO cells with or without activation.

Audit: The data, documents, and pathology materials from the 2-year studies of PETN, NF, have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* of PETN, NF, for male and female F344/N rats, based on a marginal increase in neoplasms of the Zymbal gland. Female rats might have tolerated a higher dose. There was *no evidence of carcinogenic activity* of PETN, NF, for male or female B6C3F$_1$ mice fed diets containing 25,000, or 50,000 ppm for 2 years. No nonneoplastic lesions were attributed to PETN, NF, administration.

Synonyms for PETN: 2,2-bis((nitrooxy)methyl)-1,3-propanediol dinitrate (ester); 2,2-bisdihydroxymethyl-1,3-propanediol tetranitrate; niperyt; nitropentaerythritol; pentaerythrityl tetranitrate; penthrit

Trade Names for PETN, NF: Angitet; Cardiacap; Dilcoran-80; Dipentrate; Hasethrol; Lentrat; Metranil; Mycardol; Neo-Corovas; Nitropenta; Nitropenton; Pen-

tafin; Pentanitrine; Pentitrate; Pentral 80; Pentrite; Pentritol; Pentryate; Peridex; Pergitral; Peritrate; Perityl; Prevangor; Quintrate; Subicard; Terpate; Vasodiatol

Report Date: August 1989

TR-366 Toxicology and Carcinogenesis Studies of Hydroquinone (CAS No. 123-31-9) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Hydroquinone is used an antioxidant in the rubber industry and as a developing agent in photography. It is also an intermediate in the manufacture of rubber and food antioxidants and monomer inhibitors. Hydroquinone and products containing hydroquinone are used as depigmenting agents to lighten skin. Toxicology and carcinogenesis studies were conducted by administering hydroquinone (greater than 99% pure) in corn oil or water by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Additionally, genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

Preliminary 3-day dermal studies were conducted with rats and mice using sufficient hydroquinone in 95% ethanol to crystallize on the skin (4 or 40 mg per animal); conjugated metabolites of hydroquinone were detected in the urine. Fourteen-day dermal studies were conducted at doses up to 3,840 mg/kg for rats and 4,800 mg/kg for mice. No toxic effects were seen in the 3- or 14-day dermal studies. Therefore, in further evaluations of hydroquinone, the gavage route of administration was used.

Results of Fourteen-Day and Thirteen-Week Studies: Fourteen-day gavage studies were conducted by administering hydroquinone in corn oil to rats at doses ranging from 63 to 1,000 mg/kg body weight and to mice at doses ranging from 31 to 500 mg/kg. All rats receiving 1,000 mg/kg and 1/5 male and 4/5 female rats receiving 500 mg/kg died before the end of the 14 days. Compound-related clinical signs in rats included tremors lasting up to 30 minutes after each dosing at 500 and 1,000 mg/kg. In the 14-day gavage studies with mice, 4/5 male mice and 5/5 female mice receiving 500 mg/kg and 3/5 males receiving 250 mg/kg died before the end of the studies. Tremors followed by convulsions were seen at 250 and 500 mg/kg.

In the 13-week studies, doses for rats and mice ranged from 25 to 400 mg/kg. All rats receiving 400 mg/kg and 3/10 female rats receiving 200 mg/kg died before the end of the studies. The mean body weight at necropsy of male rats administered 100 or 200 mg/kg was about 8%-9% lower than that of vehicle controls. Mean body weights of vehicle control and dosed female rats at necropsy were similar. Tremors and convulsions were observed after dosing in most rats receiving 400 mg/kg and in several female rats receiving 200 mg/kg. Inflammation and/or epithelial hyperplasia (acanthosis) of the forestomach were seen in 4/10 male rats and 1/10 female rats receiving 200 mg/kg. Toxic nephropathy, characterized by tubular cell degeneration in the renal cortex, was seen in 7/10 male and 6/10 female rats receiving 200 mg/kg and in 1/10 females receiving 100 mg/kg.

In the 13-week studies in mice, 8/10 males and 8/10 females receiving 400 mg/kg and 2/10 male mice receiving 200 mg/kg died early. Mean body weights of dosed and vehicle control mice at necropsy were similar. Liver weight to body weight ratios for dosed male mice were significantly greater than for vehicle controls. Ulceration, inflammation, or epithelial hyperplasia of the forestomach was found in 3/10 male and 2/10 female mice receiving 400 mg/kg and 1/10 females receiving 200 mg/kg.

Based on these collective results, 2-year studies were conducted by administering 0, 25, or 50 mg/kg hydroquinone in deionized water by gavage to groups of 65 rats of each sex, 5 days per week. Groups of 65 mice of each sex were administered 0, 50, or 100 mg/kg on the same schedule. Ten rats and 10 mice from each group were killed after 15 months for an interim evaluation.

Observations at Fifteen Months: In the rats killed at 15 months, the relative kidney weight for high dose male rats was greater than that for vehicle controls. The hematocrit value, hemoglobin concentration, and erythrocyte count for high dose female rats were decreased. Compound-related increased severity of nephropathy was observed in male rats. In mice killed at 15 months, the relative liver weights for high dose male and female mice were significantly greater than those for vehicle controls. Lesions seen in the liver of male mice included increased syncytial cells and diffuse cytomegaly.

Body Weights, Organ Weights, and Survival in the Two-Year Studies: Mean body weights of high dose male rats were 5%-13% lower than those of vehicle controls after week 73, and those of low dose male rats were 5%-9% lower than those of vehicle controls after week 89. Mean body weights of dosed female rats were similar to those of vehicle controls throughout the study. The relative kidney and liver weights for high dose male rats were higher than those for vehicle controls. Mean body weights of high dose male mice were 5%-8% lower than those of vehicle controls after week 93, and those of high dose female mice were 5%-14% lower after week 20. Relative liver weights were increased for dosed male and high dose female mice. No significant differences in survival were observed between any groups of rats or mice of either sex after 2 years (male rats: vehicle control, 27/55; low dose, 18/55; high dose, 18/55; female rats: 40/55; 27/55; 32/55; male mice: 33/55; 37/54; 36/55; female mice: 37/55; 39/55; 36/55).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nearly all male rats and most female rats in all vehicle control and dosed groups had nephropathy. The severity of this disease was judged to be greater in high dose male rats. Hyperplasia of the renal pelvic transitional epithelium and renal cortical cysts, changes observed with advanced renal disease, were increased in

male rats. Renal tubular hyperplasia was seen in 2 high dose male rats, and renal tubular adenomas were seen in 4/55 low dose and 8/55 high dose male rats; none was seen in vehicle controls.

Mononuclear cell leukemia in female rats occurred with a positive trend, and the incidences in the dosed groups were greater than that in the vehicle controls (vehicle control, 9/55; low dose, 15/55; high dose, 22/55). The historical incidence of leukemia in water gavage vehicle control female F344/N rats is 25% ± 15% and in untreated controls is 19% ± 7%.

Compound-related lesions observed in the liver of high dose male mice included anisokaryosis (0/55; 2/54; 12/55), syncytial alteration (5/55; 3/54; 25/55), and basophilic foci (2/55; 5/54; 11/55). The incidences of hepatocellular adenomas were increased in dosed male mice (9/55; 21/54; 20/55), but these increases were offset by decreases in the incidences of hepatocellular carcinomas (13/55; 11/54; 7/55). The incidences of hepatocellular neoplasms, primarily adenomas, were increased in dosed female mice (3/55; 16/55; 13/55).

Follicular cell hyperplasia of the thyroid gland was increased in dosed mice (male: 5/55; 15/53; 19/54; female: 13/55; 47/55; 45/55). Follicular cell adenomas were seen in 2/55 vehicle control, 1/53 low dose, and 2/54 high dose male mice and in 3/55 vehicle control, 5/55 low dose, and 6/55 high dose female mice, a follicular cell carcinoma was seen in a seventh high dose female mouse. The highest observed incidence of follicular cell adenomas or carcinomas (combined) in historical water gavage vehicle control female $B6C3F_1$ mice is 3/48 (6%).

Genetic Toxicology: Hydroquinone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. It induced trifluorothymidine (Tft) resistance in mouse L5178Y/TK lymphoma cells in the presence or absence of metabolic activation. An equivocal response was obtained in tests for induction of sex-linked recessive lethal mutations in Drosophila administered hydroquinone by feeding. Hydroquinone induced sister chromatid exchanges (SCEs) in CHO cells both with or without exogenous metabolic activation and caused chromosomal aberrations in the presence of activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of hydroquinone for male F344/N rats, as shown by marked increases in tubular cell adenomas of the kidney. There was *some evidence of carcinogenic activity* of hydroquinone for female F344/N rats, as shown by increases in mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of hydroquinone for male $B6C3F_1$ mice administered 50 or 100 mg/kg in water by gavage. There was *some evidence of carcinogenic activity* of hydroquinone for female $B6C3F_1$ mice, as shown by increases in hepatocellular neoplasms, mainly adenomas.

Administration of hydroquinone was associated with thyroid follicular cell hyperplasia in both male and female mice and anisokaryosis, multinucleated hepatocytes, and basophilic foci of the liver in male mice.

Synonyms: 1,4-benzenediol; *p*-benzenediol; benzohydroquinone; benzoquinol; 1,4-dihydroxybenzene; *p*-dihydroxybenzene; *p*-dioxobenzene; *p*-dioxybenzene; hydroquinol; hydroquinole; α-hydroquinone; *p*-hydroquinone; *p*-hydroxyphenol; quinol; β-quinol

Report Date: October 1989

TR-367 Toxicology and Carcinogenesis Studies of Phenylbutazone (CAS No. 50-33-9) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Phenylbutazone is a nonsteroidal anti-inflammatory drug. Toxicology and carcinogenesis studies were conducted by administering phenylbutazone (greater than 99% pure) in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 19 days, 13 weeks, or 2 years. Genetic toxicology studies were performed with *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells.

Nineteen-Day Studies: The deaths of 3/5 male and 4/5 female rats that received 600 mg/kg and of 2/5 females that received 300 mg/kg were considered to be chemically related. The final mean body weight of rats that received 300 or 600 mg/kg was 14%-15% or 46% lower than that of vehicle controls. No compound-related deaths occurred in mice (doses up to 600 mg/kg). The final mean body weights of dosed and vehicle control mice were similar.

Thirteen-Week Studies: Most rats that received 300 mg/kg and 1/10 male and 2/10 female rats that received 200 mg/kg died early. The final mean body weight of male rats at 300 mg/kg was 31% lower than that of the vehicle controls. The liver weight to body weight ratios were increased in the 200 and 300 mg/kg group of rats. Compound-related lesions occurred mainly in the kidney and included papillary necrosis, papillary edema, and multifocal mineralization.

Five of 10 male mice and 4/10 female mice that received 600 mg/kg died early. No other compound-related deaths occurred in mice. Final mean body weights of dosed and vehicle control mice were comparable. The liver weight to body weight ratios were increased for mice at 300 and 600 mg/kg. No compound-related histopathologic effects were observed in mice.

Body Weight and Survival in the Two-Year Studies: Two-year studies were conducted by administering 0, 50, or 100 mg/kg phenylbutazone in corn oil by gavage to groups of 50 rats of each sex, 5 days per week for 103 weeks. The doses given groups of 50 mice of each sex on the same schedule were 0, 150, or 300 mg/kg. Mean body weights of high dose rats were generally 6%-11% lower than those of vehicle controls. Mean body weights of mice were similar among all groups except for high dose female mice, which weighed 4%-11% less than vehicle controls. The survival of all groups was similar except for that of the low dose group of male rats, which was significantly lower than that of the vehicle controls at the

end of the studies; the survival of the top dose group of female rats and the vehicle control group of female mice was low but not statistically reduced (final survival—male rats: vehicle control, 33/50; low dose, 20/50; high dose, 27/50; female rats: 31/50; 35/50; 22/50; male mice: 36/50; 40/50; female mice: 22/50; 29/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Mild pyelonephritis, renal papillary necrosis, and mineralization of the renal papillae in dosed male and female rats and hyperplasia of the renal pelvis epithelium, dilatation of the renal pelvis, and renal cysts in dosed female rats were observed at increased incidences compared with those in vehicle controls. A renal tubular cell carcinoma was observed in one low dose male rat, and renal tubular adenomas were observed in three high dose male rats. A carcinoma of uncertain histogenesis was observed in one low dose female rat. Carcinomas of the renal transitional epithelium were seen in two high dose female rats. When the kidneys were step-sectioned, additional tubular cell adenomas were diagnosed in four low dose and one high dose male rats and in three low dose and one high dose female rats; none was observed in vehicle controls.

Papillomas of the transitional epithelium of the urinary bladder were seen in 2/43 low dose male and 1/49 low dose female F344/N rats. The historical incidence of urinary bladder transitional cell neoplasms in male corn oil vehicle control F344/N rats is 5/2,034 (0.2%; highest observed incidence, 2/50) and 4/2,026 (0.2%; highest observed incidence, 1/45) in females.

Adrenal medullary hyperplasia was observed at an increased incidence in high dose female rats (vehicle control, 3/50; low dose, 6/50; high dose, 19/50).

Ulcers of the forestomach were observed at increased incidences in high dose rats (male: 0/50; 5/50; 6/50; female: 2/49; 1/49; 12/49). In high dose female rats, acanthosis (4/49; 0/49; 12/49), hyperkeratosis (3/49; 0/49; 12/49), and basal cell hyperplasia (4/49; 1/49; 12/49) of the forestomach were observed at increased incidences. No neoplasms were associated with these stomach lesions.

Peliosis hepatis, centrilobular cytomegaly and karyomegaly, fatty change, hepatocellular degeneration, and coagulative necrosis of the liver were observed in dosed male mice; clear cell foci were observed in five high dose male mice. The incidences of hepatocellular adenomas and adenomas or carcinomas (combined) in male mice were increased in the high dose group (adenomas or carcinomas, combined: 16/50; 14/50; 31/50).

Genetic Toxicology: Phenylbutazone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with or without exogenous metabolic activation. Phenylbutazone produced a positive response in the mouse lymphoma assay in both the presence and absence of activation. Phenylbutazone induced chromosomal aberrations in CHO cells in the presence, but not the absence, of exogenous metabolic activation; no induction of sister chromatid exchanges was observed in CHO cells in the presence or absence of activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of phenylbutazone for male F344/N rats, as shown by the occurrence of small numbers of renal tubular cell adenomas or carcinomas. There was *some evidence of carcinogenic activity* for female F344/N rats, as shown primarily by the occurrence of two rare transitional cell carcinomas in the top dose group; none has ever been seen in vehicle control or untreated control female rats. Tubular cell adenomas may have been associated with the administration of phenylbutazone to female rats. There was *some evidence of carcinogenic activity* for male $B6C3F_1$ mice, as shown by the increased incidence of hepatocellular adenomas or carcinomas (combined). There was *no evidence of carcinogenicity* for female $B6C3F_1$ mice administered phenylbutazone in corn oil by gavage at doses of 150 or 300 mg/kg.

Phenylbutazone was also nephrotoxic to rats, as shown by the dose-related increase in the severity of age-related nephropathy, necrosis of the renal papilla, and mineralization of the collecting ducts in the papilla.

Synonyms: 4-butyl-1,2-diphenyl-3,5-pyrazolidinedione; 3,5-dioxo-1,2-diphenyl-4-*n*-butylpyrazolidine

Trade Names: There have been over 100 registered trade names including: Anerval; Azobutil; Bizolin 200; Butacote; Butadion; Butagesic; Butazolidin; Chembutazone; Equi Bute; Flexazone; Fenibutol; G 13,871; Pyrazolidin; Reumazol; Robizon-V; Uzone

Report Date: March 1990

TR-368 Toxicology and Carcinogenesis Studies of Nalidixic Acid (CAS No. 389-08-2) in F344/N Rats and $B6C3F_1$ Mice (Feed Studies)

Nalidixic acid is an antimicrobial agent to treat bacterial infections of the urinary tract. Toxicology and carcinogenesis studies were conducted by feeding diets containing nalidixic acid (approximately 99% pure) to groups of F344/N rats and $B6C3F_1$ mice of each sex for 13 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Thirteen-Week Studies: Nalidixic acid was administered at dietary concentrations ranging from 1,000 to 16,000 ppm. One female rat that received 16,000 ppm nalidixic acid died before the end of the studies; no other compound-related deaths occurred in rats and mice. The final mean body weights of rats that received 8,000 or 16,000 ppm were 23% or 49% lower than those of controls for males and 11% or 31% lower for females. Feed consumption by rats receiving 16,000 ppm was approximately two-thirds that by controls. Liver weight to body weight ratios for male rats that received 2,000 ppm or more and female rats that received 8,000 ppm or more were significantly greater than those for controls. Degeneration of the germinal epithelium in the semi-

niferous tubules of the testis was observed in 10/10 male rats that received 16,000 ppm; no other compound-related histopathologic effects were observed in rats. The final mean body weights of mice that received 8,000 or 16,000 ppm were 10%-20% lower than those of controls. Feed consumption by dosed mice was similar to that by controls. Liver weight to body weight ratios were significantly greater for male mice receiving 2,000, 8,000, or 16,000 ppm and for female mice receiving 4,000, 8,000, or 16,000 ppm than for the controls. No compound-related histopathologic effects were observed in mice.

Based on these results, 2-year studies of nalidixic acid were conducted by feeding diets containing 0, 2,000, or 4,000 ppm nalidixic acid to groups of 50 male and 50 female F344/N rats and 50 male and 50 female $B6C3F_1$ mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose rats were 7%-23% lower than those of controls, and those of low dose male rats were 6%-11% lower than those of controls. The average daily feed consumption by dosed rats ranged from 89% to 96% that by controls. The average amount of nalidixic acid consumed per day was approximately 80 or 175 mg/kg for low dose or high dose rats. Mean body weights of high dose male mice were 1%-8% lower than those of controls throughout the study. Mean body weights of dosed female mice were 5%-17% lower than those of controls. Average daily feed consumption by dosed mice was within 3% of that by controls. The estimated average amount of nalidixic acid consumed per day was approximately 220 or 475 mg/kg for low dose or high dose mice. No significant differences in survival were seen between any groups of rats or mice of either sex after 2 years (male rats: control, 27/50; low dose, 28/50; high dose, 27/50; female rats: 22/50; 31/50; 29/50; male mice: 33/50; 34/50; 31/50; female mice: 40/50; 43/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidences of preputial gland neoplasms in dosed male rats and of clitoral gland neoplasms in dosed female rats were significantly greater than those in controls (male—preputial gland adenomas, papillomas, or carcinomas, combined: control, 3/49; low dose, 19/49; high dose, 20/47; female—clitoral gland adenomas, papillomas, or carcinomas, combined: 5/46; 15/46; 16/47).

A squamous cell carcinoma of the tongue was seen in two high dose male rats. The historical incidence of oral cavity neoplasms in untreated control male F344/N rats is 7/1,596 (0.4%).

There were decreased incidences of leukemia (20/50; 9/50; 7/50) and mammary gland neoplasms (10/50; 7/50; 2/50) in dosed female rats and of pituitary gland neoplasms (11/49; 2/50; 2/50) in dosed male rats.

Retinal degeneration and cataracts of the eye were observed at increased incidences in dosed rats (degeneration—male: 4/48; 41/48; 47/49; female: 2/47; 40/48; 46/50; cataracts—male: 11/48; 23/48; 38/49; female: 0/47; 18/48; 14/50). The cause of these cataracts and retinal degeneration is uncertain because cages were not rotated and low and high dose groups of rats may have been exposed to greater light intensity than were the controls.

Subcutanous tissue fibrosarcomas and fibromas or fibrosarcomas (combined) were increased in dosed male mice (fibromas or fibrosarcomas, combined: 5/50; 9/50; 14/50). There were no increased incidences of neoplasms in dosed female mice.

Genetic Toxicology: Nalidixic acid was not mutagenic in any of several in vitro short-term tests. No gene reversion was observed in *S. typhimurium* strains TA97, TA98, TA100, or TA1535 after exposure to nalidixic acid in either the presence or absence of exogenous metabolic activation. Results of tests for induction of trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells were negative with or without metabolic activation. In CHO cells, nalidixic acid did not induce sister chromatid exchanges or chromosomal aberrations in either the presence or absence of activation.

Conclusions: Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity* of nalidixic acid for F344/N rats, as indicated by increased incidences of preputial gland neoplasms in males and clitoral gland neoplasms in females. There was *equivocal evidence of carcinogenic activity* for male $B6C3F_1$ mice fed diets containing nalidixic acid, as indicated by marginally increased incidences of subcutaneous tissue neoplasms. There was *no evidence of carcinogenic activity* for female $B6C3F_1$ mice fed diets containing 2,000 or 4,000 ppm nalidixic acid for 2 years.

Synonym: 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid

Trade Names: NegGram®; Dixiben®; Nalidixan®; Nalurin®; Nogram®; UroNeg®; Uralgin®; Urisal®

Report Date: October 1989

TR-369 Toxicology and Carcinogenesis Studies of α-Methylbenzyl Alcohol (CAS No. 98-85-1) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Toxicology and carcinogenesis studies of α-methylbenzyl alcohol (greater than 99% pure), a cosmetic ingredient and food flavoring agent, were conducted by administering the chemical in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells. α-Methylbenzyl alcohol was nominated for study by the National Cancer Institute because of the potential for widespread human exposure.

Sixteen-Day and Thirteen-Week Studies: The doses used in the 16-day studies for rats and mice ranged between 125 and 2,000 mg/kg. Six of 10 rats and all mice dosed at 2,000 mg/kg died. In addition, because 7/9 mice dosed at 1,000 mg/kg died, the doses selected for the 13-week studies for mice (47-750 mg/kg) were half those used for rats (93-1,500 mg/kg).

In the 13-week studies, deaths of 1/10 male and 3/10 female rats dosed at 1,500 mg/kg were compound related; none of the mice died. Body weight gain was reduced in rats at 1,500 mg/kg; there were no significant histopathologic lesions in either rats or mice. The only compound-related effects were ataxia, labored breathing, and lethargy for up to 30 minutes after dosing in rats and mice given the two highest doses and increases in liver weight to body weight ratios for male rats given the three highest doses and for female rats at all doses.

Based on the pattern of mortality and the effects on body weight gain in the short-term studies, doses of 375 and 750 mg/kg α-methylbenzyl alcohol were administered in corn oil by gavage, 5 days per week for 103 weeks, to groups of 50 rats and 50 mice of each sex.

Two-Year Studies: Significant reduction in body weight gain commenced at weeks 20-30 in high dose male and female rats, and body weights were 20%-30% below those of vehicle controls at study termination. In the low dose groups, body weight reduction occurred only in male rats during the last 10 weeks of the study. After 80 weeks, 60% of the high dose rats and 80%-100% of the low dose and vehicle control rats were alive; thereafter, the number of deaths in the chemically exposed groups increased sharply so that, at the end of 2 years, final survival for vehicle control, low dose, and high dose rats was 35/50; 8/50; and 1/50 for males and 34/50, 25/50, and 11/50 for females. There were a large number of gavage accidents in these studies (1, 9, and 8 for male rats and 1, 4, and 14 for female rats), but these accidents did not contribute to the increase in mortality after week 80, as all but 4 of these occurred earlier.

Mortality in the last quarter of the study was thought to be due to the effects of cumulative toxicity of α-methylbenzyl alcohol on a renal excretory system already compromised by aging. Renal nephropathy that commonly occurs during aging was found in all groups of rats, but the severity was greater in male rats dosed with α-methylbenzyl alcohol. In addition, a collection of nonneoplastic lesions (parathyroid hyperplasia, calcification of the heart and glandular stomach, and fibrous osteodystrophy of bone) was found in the dosed male rats; these lesions were probably secondary to mineral imbalance arising from renal dysfunction.

Since survival was poor in low and high dose male and high dose female rats, the sensitivity of the study for detecting a carcinogenic effect in these groups was reduced. Despite this limitation, there were dose-related increases in the incidences of renal tubular cell adenomas or adenocarcinomas (combined) in male rats (vehicle control, 0/50; low dose, 2/50; high dose, 5/50). In addition, transitional cell papillomas of the urinary bladder were observed in one high dose male and two high dose female rats.

In mice, a reduction in body weight gain was apparent in the high dose groups of males and females. Final survival rates in mice were similar among groups (male: 39/49; 40/50; 28/50; female: 41/50; 41/50; 38/50). No neoplastic or nonneoplastic lesions were attributed to α-methylbenzyl alcohol administration in mice of either sex.

Genetic Toxicology: α-Methylbenzyl alcohol was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in the presence or absence of exogenous metabolic activation. α-Methylbenzyl alcohol produced a positive response without activation in the mouse L5178Y/TK$^{+/-}$ lymphoma assay for induction of trifluorothymidine resistance; it was not tested with activation. In cytogenetic tests with CHO cells, α-methylbenzyl alcohol induced chromosomal aberrations in the presence, but not the absence, of metabolic activation; no induction of sister chromatid exchanges was observed in CHO cells after exposure to α-methylbenzyl alcohol.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of α-methylbenzyl alcohol for male F344/N rats, as shown by increased incidences of renal tubular cell adenomas and adenomas or adenocarcinomas (combined). There was *no evidence of carcinogenic activity* for female F344/N rats administered 375 or 750 mg/kg. Renal toxicity characterized by severe nephropathy and related secondary lesions was observed in the dosed rats, and excessive mortality occurred during the last quarter of the studies. Poor survival reduced the sensitivity of the studies for detecting the presence of a carcinogenic response both in chemically exposed groups of male rats and in the high dose group of female rats. There was *no evidence of carcinogenic activity* of α-methylbenzyl alcohol for male or female B6C3F$_1$ mice administered 375 or 750 mg/kg for 2 years.

Synonyms: styrallyl alcohol; styralyl alcohol; α-methylbenzenemethanol; phenylmethylcarbinol; 1-phenethyl alcohol

Report Date: January 1990

TR-370 Toxicology and Carcinogenesis Studies of Benzofuran (CAS No. 271-89-6) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Benzofuran is used as an intermediate in the polymerization of coumarone-indene resins found in various corrosion-resistant coatings such as paints and varnishes, in water-resistant coatings for paper products and fabrics, and in adhesives approved for use in food containers. Toxicology and carcinogenesis studies were conducted by administering benzofuran (approximately 99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology tests were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: Benzofuran doses for groups of five rats ranged from 63 to 1,000 mg/kg and from 16 to 250 mg/kg for mice. All male and female rats that received 1,000 mg/kg and one female rat that received 500 mg/kg died before the end of the studies. The final mean

body weights of male rats that received 250 or 500 mg/kg were 13% or 21% lower than that of controls; the final mean body weight of female rats that received 500 mg/kg was 10% lower than that of controls. Final mean body weights of chemically exposed and control mice were similar. No compound-related histologic lesions were found in rats or mice.

Thirteen-Week Studies: Doses for groups of 10 rats and 10 mice ranged from 31 to 500 mg/kg. One female rat that received 500 mg/kg and one that received 250 mg/kg died before the end of the study. Final mean body weights of male rats that received 125, 250, or 500 mg/kg were 11%, 17%, or 27% lower than that of vehicle controls; the final mean body weight of female rats that received 500 mg/kg was 11% lower than that of vehicle controls. Histologic lesions observed in chemically exposed rats included minimal hepatocellular necrosis, increased severity of nephropathy, and cytoplasmic vacuolization of the adrenal cortex.

Seven male and three female mice that received 500 mg/kg and one male mouse that received 250 mg/kg died before the end of the 13-week studies. The final mean body weight of mice that received 500 mg/kg was 13% lower than that of vehicle controls. Nephrosis was observed in male mice that received 250 mg/kg.

Based on reduced mean body weights, increased severity of nephropathy, and hepatocellular necrosis, benzofuran doses selected for the 2-year studies in rats were 30 or 60 mg/kg for males and 60 or 120 mg/kg for female. Based on increased mortality and nephrosis in male mice, doses selected for the 2-year studies in mice were 60 or 120 mg/kg for males and 120 or 240 mg/kg for females.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose rats and dosed male mice were 4%-11% lower than those of vehicle controls. Mean body weights of chemically exposed female mice were 8%-35% lower than those of vehicle controls. The survival of chemically exposed male rats was reduced after week 92 (survival at week 89: vehicle control, 47/50; low dose, 39/50; high dose, 38/50; final survival: vehicle control, 33/50; low dose, 12/50; high dose, 18/50). Survival of chemically exposed female rats and male mice was similar to that of vehicle controls after 2 years (female rats: 27/50; 23/50; 25/50; male mice: 33/50; 20/50; 28/50). Deaths of 10 low dose male mice at weeks 20-21 were caused by a dosing error; these animals were not included in survival and tumor analyses. Survival of chemically exposed female mice was reduced after week 89 (final survival: 37/50; 19/50; 21/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy occurred with increased severity in chemically exposed male rats. The incidences of parathyroid hyperplasia, fibrous osteodystrophy, mineralization of the pulmonary artery, renal cortical cysts, and hyperplasia of the pelvic epithelium were increased in chemically exposed male rats. The incidence of nephropathy was increased in chemically exposed female rats (vehicle control, 29/50; low dose, 48/50; high dose, 39/50). Renal atypical tubular cell hyperplasia and renal tubular cell adenocarcinomas occurred in chemically exposed female rats (atypical tubular cell hyperplasia: 0/50; 1/50; 3/50; tubular cell adenocarcinomas: 0/50; 1/50; 4/50). No renal tubular cell adenocarcinomas have been observed in 2,094 female corn oil vehicle control F344/N rats in National Toxicology Program studies.

Chronic inflammation, ulcers, and epithelial hyperplasia of the forestomach were observed at increased incidences in chemically exposed male rats (chronic inflammation: 1/50; 11/50; 6/49; ulcers: 1/50; 5/50; 8/49; epithelial hyperplasia: 9/50; 15/50; 18/49).

Metaplastic hepatocytes arising within pancreatic islets occurred at an increased incidence in high dose female rats (0/50; 1/50; 11/49).

The incidences of neurilemomas were markedly increased above the historical control incidences (0.1%-0.4%) in all groups of rats (male: 18/50; 13/50; 14/50; female: 7/50; 9/50; 3/50).

Syncytial alteration of the liver occurred at increased incidences in male mice exposed to benzofuran. The incidences of hepatocellular adenomas, hepatoblastomas (high dose male mice) and hepatocellular adenomas, hepatocellular carcinomas, or hepatoblastomas (combined) were increased in chemically exposed mice (male—adenomas: 4/49; 24/39; 34/48; hepatoblastomas: 0/49; 3/39; 18/48; carcinomas, adenomas, or hepatoblastomas, combined: 12/49; 31/39; 40/48; female—adenomas: 1/50; 22/48; 21/47; hepatoblastomas: 0/50; 1/48; 2/47; carcinomas, adenomas, or hepatoblastomas, combined: 4/50; 25/48; 22/47).

Squamous cell papillomas or carcinomas (combined) of the forestomach were increased in chemically exposed mice (male: 2/49; 11/39; 13/48; female: 2/50; 9/50; 5/50).

The incidences of epithelial hyperplasia of the bronchioles were increased in chemically exposed mice. The incidences of alveolar/bronchiolar adenomas or carcinomas (combined) in high dose males and chemically exposed females were increased (adenomas or carcinomas, combined—male: 10/49; 9/39; 19/48; female: 2/50; 9/48; 14/47).

Genetic Toxicology: Benzofuran was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of exogenous metabolic activation. Benzofuran induced trifluorothymidine resistance in mouse L5178Y lymphoma cells treated in the absence of metabolic activation; this assay was not conducted with activation. Benzofuran induced sister chromatid exchanges but not chromosomal aberrations in CHO cells in the presence and absence of activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of benzofuran for male F344/N rats receiving doses of 30 or 60 mg/kg per day. There was *some evidence of carcinogenic activity* of benzofuran for female F344/N rats, based on increased incidences of tubular cell adenocarcinomas of the kidney. There was *clear evidence of carcinogenic activity* for male and female $B6C3F_1$ mice, based on increased incidences of neoplasms of the liver, lung, and forestomach.

Exposure to benzofuran increased the severity of nephropathy in male rats, increased the incidences of

nephropathy in female rats, and induced hepatocellular metaplasia in the pancreas in female rats. Nonneoplastic lesions observed in mice exposed to benzofuran included syncytial alteration of the liver, bronchiolar epithelial hyperplasia, and epithelial hyperplasia of the forestomach.

Synonyms: coumarone; cumarone

Report Date: October 1989

TR-371 Toxicology and Carcinogenesis Studies of Toluene (CAS No. 108-88-3) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

Toluene is used to back-blend gasoline, as a chemical intermediate, and as a solvent; 920 million gallons were produced in the United States in 1988. Toxicology studies were conducted by administering toluene (greater than 99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 13 weeks or by whole-body inhalation exposure for 14 or 15 weeks. Toxicology and carcinogenesis studies were conducted by whole-body inhalation exposure of F344/N rats and B6C3F$_1$ mice of each sex for 15 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y lymphoma cells, and Chinese hamster ovary cells.

Thirteen-Week Gavage Studies: All rats that received the top dose of 5,000 mg/kg died during the first week, and 8/10 male rats that received 2,500 mg/kg died early. The final mean body weight of male rats that received 2,500 mg/kg was 19% lower than that of vehicle controls. Relative liver, kidney, and heart (female only) weights for rats that received the higher doses were greater than those for vehicle controls. Necrosis of the brain and hemorrhage of the urinary bladder were seen at increased incidences in dosed rats.

All mice that received the top dose of 5,000 mg/kg died during the first week, and 40% of those that received 2,500 mg/kg died before the end of the 13-week gavage studies. The final mean body weight of males at 2,500 mg/kg was 16% lower than that of vehicle controls. At the higher doses, relative liver weights were increased for mice.

Fifteen-Week and Fourteen-Week Inhalation Studies: Eight of 10 male rats exposed at the top exposure concentration of 3,000 ppm died during week 2. Final mean body weights of rats exposed at concentrations of 2,500 or 3,000 ppm were 14%-25% lower than that of controls. As in the gavage studies, the relative liver, kidney, and heart weights for rats exposed at the top two concentrations were increased compared with those for controls. No compound-related effects were seen on sperm; no adverse effects on the estrous cycle were observed.

Five of 10 male mice and all female mice exposed at 3,000 ppm and 70% of female mice at 2,500 ppm died during the first 2 weeks. Final mean body weights of all exposed groups were 7%-13% lower than those of controls. Relative liver weights for mice exposed at 625 ppm or higher, relative lung weights for mice exposed at 1,250 ppm or higher, and relative kidney weights for female mice exposed at 1,250 ppm or higher were greater than those for controls. Centrilobular hypertrophy of the liver was observed in all male mice exposed at 2,500 ppm and 70% of male mice exposed at 3,000 ppm. No effects on sperm or the estrous cycle were observed.

Fifteen-Month and Two-Year Inhalation Studies: Long-term studies were conducted by exposing groups of 60 rats of each sex to 0, 600, or 1,200 ppm toluene by inhalation, 6.5 hours per day, 5 days per week. Groups of 60 mice of each sex were exposed at 0, 120, 600, or 1,200 ppm on the same schedule. Ten animals per group (except male mice) were removed for toxicologic evaluation after being exposed for 15 months. All other animals were exposed to toluene for 103 weeks.

In the 15-month inhalation studies, the incidences and severity of nonneoplastic lesions of the nasal cavity (degeneration of olfactory and respiratory epithelium and goblet cell hyperplasia) were increased in exposed rats. Minimal hyperplasia of the bronchial epithelium was seen in 4/10 female mice at 1,200 ppm. The severity of nephropathy was slightly increased in exposed female rats. No chemical-induced neoplasms were observed.

Body Weight and Survival in the Two-Year Studies: Mean body weights of rats and mice were generally similar (yearly averages within 5%) among groups throughout the 2-year studies. No significant differences in survival were observed among rats or mice of either sex, although survival in all groups of male mice was lower than usual (male rats: control, 30/50; 600 ppm, 28/50; 1,200 ppm, 22/50; female rats: 33/50; 35/50; 30/50; male mice: control, 17/60; 120 ppm, 22/60; 600 ppm, 16/60; 1,200 ppm, 19/60; female mice: 30/50; 33/50; 24/50; 32/50). Scrotal, preputial, and penile lesions observed in male mice were associated with many of the early deaths and with animals killed in a moribund condition.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Nephropathy was seen in almost all rats, and the severity was somewhat increased in exposed rats. A rare renal tubular cell carcinoma in a female rat and an equally uncommon sarcoma of the kidney in another female rat were seen in the 1,200-ppm exposure group. Erosion of the olfactory epithelium and degeneration of the respiratory epithelium were increased in exposed rats. Inflammation of the nasal mucosa and metaplasia of the olfactory epithelium were increased in exposed female rats. A rare squamous cell carcinoma of the nasal mucosa was seen in one female rat at 1,200 ppm. A squamous cell papilloma of the forestomach was observed in one female rat at 1,200 ppm, and a squamous cell carcinoma was observed in a second female rat at 1,200 ppm. No chemically related neoplasms were found in male rats, and the one nasal, two kidney, and two forestomach neoplasms observed in female rats were considered not to be associated with inhalation exposed to toluene.

For mice, no biologically important increases were observed for any nonneoplastic or neoplastic lesions.

Genetic Toxicology: Toluene did not induce gene mutations in *S. typhimurium* strain TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. In the mouse lymphoma assay, toluene gave an equivocal response with and without exogenous metabolic activation. Toluene did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* for male or female F344/N rats exposed to toluene at concentrations of 600 or 1,200 ppm. There was *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice exposed by inhalation to toluene at concentrations of 120, 600, or 1,200 ppm for 2 years.

Synonyms: monomethylbenzene; methylbenzene; toluol; phenylmethane; tolueen (Dutch); toluen (Czech), tolueno (Spanish); toluolo (Italian)

Trade Name: Methacide

Report Date: February 1990

TR-372 Toxicology and Carcinogenesis Studies of 3,3'-Dimethoxybenzidine Dihydrochloride (CAS No. 20325-40-0) in F344/N Rats (Drinking Water Studies)

3,3'-Dimethoxybenzidine dihydrochloride is an off-white powder with a melting point of 274° C. 3,3'-Dimethoxybenzidine is used principally as an intermediate in the production of commercial bisazobiphenyl dyes for coloring textiles, paper, plastic, rubber, and leather. In the synthesis of the bisazobiphenyl dyes, the amine groups of 3,3'-dimethoxybenzidine are chemically linked with other aromatic amines. A small quantity of 3,3'-dimethoxybenzidine is also used as an intermediate in the production of *o*-dianisidine diisocyanate, which is used in isocyanate-based adhesive systems and as a component of polyurethane elastomers.

3,3'-Dimethoxybenzidine dihydrochloride was evaluated in toxicity and carcinogenicity studies as part of the National Toxicology Program's Benzidine Dye Initiative. This Initiative was designed to evaluate the representative benzidine congeners and benzidine congener-derived and benzidine-derived dyes. 3,3'-Dimethoxybenzidine dihydrochloride was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering 3,3'-dimethoxybenzidine dihydrochloride (greater than 97.5% pure) in drinking water to groups of F344/N rats of each sex for 14 days, 13 weeks, 9 months, or 21-months. The 21-month studies were intended to last 24 months but were terminated early because of rapidly declining survival due to neoplasia. Studies were performed only in rats because similar studies are being performed in mice at the National Center for Toxicology Research. Genetic toxicology studies were conducted with *Salmonella typhimurium*, Chinese hamster over (CHO) cells, and *Drosophila melanogaster*.

Fourteen-Day Studies: All rats receiving drinking water concentrations up to 4,500 ppm lived to the end of the studies. Rats that received water containing 4,500 ppm 3,3'-dimethoxybenzidine dihydrochloride lost weight. Water consumption decreased with increasing concentration of chemical and at 4,500 ppm was less than one-fourth that by the controls. Lymphoid depletion of the thymus in males and hypocellularity of the bone marrow in males and females were seen at the 4,500-ppm concentration, but not at the next lower concentration or in controls.

Thirteen-Week Studies: All rats receiving concentrations up to 2,500 ppm lived to the end of the studies. Final mean body weights of rats given drinking water containing 1,250 or 2,500 ppm 3,3'-dimethoxybenzidine dihydrochloride were 5%-20% lower than those of controls. Water consumption at these concentrations was 40%-60% that consumed by controls. Compound-related effects in rats given water containing 2,500 ppm 3,3'-dimethoxybenzidine dihydrochloride included a mind exacerbation of naturally occurring nephropathy and the presence of a yellow-brown pigment (lipofuscin) in the cytoplasm of thyroid follicular cells. Serum triiodothyronine (T_3) and thyroxin (T_4) concentrations in females receiving 330 ppm or more and T_4 concentrations in males receiving 170 ppm or more were significantly lower than in controls. Thyrotropin (TSH) concentrations were comparable in controls and exposed rats.

Based on the chemical-related nephropathy and reductions in water consumption and body weight gain observed in the 13-week studies, doses for the long-term studies in male and female rats were 0 or 330 ppm 3,3'-dimethoxybenzidine dihydrochloride in drinking water administered for 9 months and 0, 80, 170, or 330 ppm administered for 21 months.

Nine-Month Studies: Ten rats of each sex in control and 330-ppm groups were evaluated after 9 months. Significant decreases in T_3 and T_4 concentrations were seen in exposed male and female rats. Other lesions seen in exposed rats included foci of alteration in the liver, a carcinoma of the preputial gland in one male, a carcinoma of the clitoral gland in one female, and carcinoma of the Zymbal gland in two males.

Body Weights and Survival in the Twenty-One-Month Studies: The average amount of 3,3'-dimethoxybenzidine dihydrochloride consumed per day was approximately 6, 12, or 21 mg/kg for low, mid, or high dose male rats and 7, 14, or 23 mg/kg for low, mid, or high dose female rats. Mean body weights of male and female rats began to decrease relative to those of controls after about 1 year of exposure at 170 or 330 ppm and were 6%-22% lower for males and 7%-17% lower for females. Survival of rats exposed to 3,3'-dimethoxybenzidine dihydrochloride was reduced because animals were dying with neoplasms or being killed in a moribund condition (survival at 21

months—male: control, 44/60, 73%; low dose, 8/45, 18%; mid dose, 0/75; high dose, 0/60; female: 45/60, 75%; 15/45, 33%; 6/75, 8%; 0/60). Because of these early compound-related deaths, the studies were terminated at 21 months.

Nonneoplastic and Neoplastic Effects in the Twenty-One-Month Studies: Increased incidences of several nonneoplastic lesions were observed in exposed rats, including hematopoietic cell proliferation in the spleen and cystic and centrilobular degeneration and necrosis of the liver. Neoplasms attributed to 3,3'-dimethoxybenzidine dihydrochloride exposure were observed in rats at many tissue sites, including the skin, Zymbal gland, preputial and clitoral glands, oral cavity, small and large intestines, liver, brain, mesothelium, mammary gland, and uterus/cervix. The incidences of these neoplasms in male and female rats are given in the abstract summary table (see page 5 of the Technical Report).

Genetic Toxicology: 3,3'-Dimethoxybenzidine was mutagenic in *S. typhimurium* strain TA100 with exogenous metabolic activation and in strain TA98 without activation; a weakly positive response was observed in strain TA1535 with metabolic activation. 3,3'-Dimethoxybenzidine induced sister chromatid exchanges and chromosomal aberrations in CHO cells with and without exogenous metabolic activation. 3,3'-Dimethoxybenzidine did not induce sex-linked recessive lethal mutations in adult male *D. melanogaster* exposed via feeding or injection.Conclusions: Under the conditions of these 21-month drinking water studies, there was *clear evidence of carcinogenic activity* of 3,3'-dimethoxybenzidine dihydrochloride for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal gland, preputial gland, oral cavity, intestine, liver, and mesothelium. Increased incidences of astrocytomas of the brain may have been related to chemical administration. There was *clear evidence of carcinogenic activity* of 3,3'-dimethoxybenzidine dihydrochloride for female F344/N rats, as indicated by benign and malignant neoplasms of the Zymbal gland, clitoral gland, and mammary gland. Increases in neoplasms of the skin, oral cavity, large intestine, liver, and uterus/cervix were also considered to be related to chemical administration of 3,3'-dimethoxybenzidine dihydrochloride.

Synonyms: *o*-dianisidine dihydrochloride; 3,3'-dimethoxy-1,1-biphenyl)-4,4'-diamine dihydrochloride; 3,3'-dimethoxy-4,4'-diaminobiphenyl dihydrochloride

Report Date: January 1990

TR-373 Toxicology and Carcinogenesis Studies of Succinic Anhydride (CAS No. 108-30-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Succinic anhydride, a food additive, is also used in the manufacture of polymeric materials, pharmaceuticals, and agricultural and industrial chemicals. Toxicology and carcinogenesis studies were conducted by administering suspensions of succinic anhydride (97% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 or 20 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Twenty-Day or Sixteen-Day and Thirteen-Week Studies: In the 20-day studies in rats, doses of succinic anhydride given on 14 exposure days ranged from 47 to 750 mg/kg. Compound-related deaths occurred in the groups of males that received 375 mg/kg or higher doses and in females that received 187 mg/kg or higher doses. Necrosis and inflammation of the upper respiratory tract were seen in 3/10 male and 3/10 female rats given 750 mg/kg and 2/10 female rats given 375 mg/kg.

In the 16-day studies in mice, doses of succinic anhydride given on 12 exposure days ranged from 219 to 3,500 mg/kg. All animals that received 875 mg/kg or higher doses of succinic anhydride died before the end of the studies. No compound-related lesions were seen in male or female mice examined from the 438 mg/kg dose group.

In the 13-week studies in rats, doses of succinic anhydride ranged from 25 to 400 mg/kg for males and from 12.5 to 200 mg/kg for females. Deaths of 8/10 male rats that received 400 mg/kg and 4/10 males and 5/10 females that received 200 mg/kg were compound related. At necropsy, the mean body weights of male rats that received 200 or 400 mg/kg were 9% or 15% lower than that of vehicle controls, whereas the mean body weights of dosed and vehicle control female rats were similar. No compound-related gross or microscopic lesions were observed.

In the 13-week studies in mice, doses of succinic anhydride ranged from 37 to 600 mg/kg. All 10 males and 8/10 females that received 600 mg/kg and 2/10 males and 2/10 females that received 300 mg/kg died before the end of the studies. The final mean body weights of mice that received 150 or 300 mg/kg were 13% or 9% lower than that of vehicle controls for males and 8% or 7% lower for females. Mild inflammation of the stomach was observed in 7/10 male mice that received 150 mg/kg and 5/10 males that received 300 mg/kg compared with 2/10 vehicle controls.

Based primarily on the effects of administration of succinic anhydride on survival and mean body weights of rats and mice, doses for the 2-year studies were 0, 50, or 100 mg/kg to groups of 60 rats of each sex; 0, 38, or 75 mg/kg to groups of 50 male mice; and 0, 75, or 150 mg/kg to groups of 50 female mice. Succinic anhydride was administered as a suspension in corn oil by gavage, 5 days per week for 103 weeks.

Body Weights and Survival in the Two-Year Studies: Mean body weights of high dose rats were 5%-11% lower than those of vehicle controls during the second year of the studies. No significant differences in survival after 2 years were observed between any groups of rats of either sex (male: vehicle control, 36/60; low dose, 33/60; high dose, 32/60; female: 31/60; 27/60; 27/60). For mice, mean body weights of high dose males were generally 5%-12% lower than those of vehicle controls throughout the study.

Mean body weights of high dose female mice were 10%-32% lower than those of vehicle controls; mean body weights of low dose female mice were 10%-20% lower than those of vehicle controls. The survival of high dose male mice was significantly greater than that of vehicle controls after week 77 (survival after 2 years—male: 27/50; 30/50; 42/50; female: 37/50; 38/50; 41/50). No other differences in survival were observed between any groups of mice of either sex.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: At no site in rats or mice was there a chemical-related increase in the incidence of nonneoplastic or neoplastic lesions. A sufficient number of animals in each dose group lived long enough to allow evaluation of the potential carcinogenicity of succinic anhydride.

Genetic Toxicology: Succinic anhydride was not mutagenic in *S. typhimurium* with or without exogenous metabolic activation. The chemical did not induce sister chromatid exchanges or chromosomal aberrations in cultured CHO cells in the presence or absence of exogenous metabolic activation.

Conclusions: Under the conditions of these 2-year studies, there was *no evidence of carcinogenic activity* of succinic anhydride for male or female F344/N rats given 50 or 100 mg/kg succinic anhydride. There was *no evidence of carcinogenic activity* for male $B6C3F_1$ mice given 38 or 75 mg/kg succinic anhydride or for female $B6C3F_1$ mice given 75 or 150 mg/kg.

Synonyms: butanedioic anhydride; dihydro-2,5-furandione; 2,5-diketotetrahydrofuran; succinic acid anhydride; succinyl anhydride; succinyl oxide; tetrahydro-2,5-dioxofuran

Report Date: January 1990

TR-374 Toxicology and Carcinogenesis Studies of Glycidol (CAS No. 556-52-5) In F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Glycidol is a viscous liquid that is used as a stabilizer in the manufacture of vinyl polymers, as an additive for oil and synthetic hydraulic fluids, and as a diluent in some epoxy resins. Toxicology and carcinogenesis studies were conducted by administering glycidol (94% pure, containing 1.2% 3-methoxy-1,2-propanediol, 0.4% 3-chloro-1,2-propanediol, 2.8% diglycidyl ether, and 1.1% 2,6-dimethanol-1,4-dioxane) in water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells, *Drosophila melanogaster*, and the bone marrow of male $B6C3F_1$ mice.

Sixteen-Day Studies: Glycidol doses for groups of five rats or five mice of each sex ranged from 37.5 to 600 mg/kg; vehicle controls received distilled water. All rats that received 600 mg/kg died between days 3 and 13. Edema and degeneration of the epididymal stroma, atrophy of the testis, and granulomatous inflammation of the epididymis occurred in males that received 300 mg/kg.

All mice that received 600 mg/kg and two males and two females that received 300 mg/kg died by day 4 of the studies. Focal demyelination in the medulla and thalamus of the brain occurred in all female mice that received 300 mg/kg.

Thirteen-Week Studies: Doses for groups of 10 rats ranged from 25 to 400 mg/kg, and doses for groups of 10 mice ranged from 19 to 300 mg/kg; vehicle controls received distilled water. All rats that received 400 mg/kg died by week 2; three males and one female that received 200 mg/kg died during weeks 11-12. Final mean body weights of male rats that received 50, 100, or 200 mg/kg were 96%-85% that of vehicle controls; final mean body weights of female rats receiving the same doses were 95%-89% that of vehicle controls. Sperm count and sperm motility were reduced in male rats that received 100 or 200 mg/kg. Necrosis of the cerebellum, demyelineation in the medulla of the brain, tubular degeneration and/or necrosis of the kidney, lymphoid necrosis of the thymus, and testicular atrophy and/or degeneration occurred in rats that received 400 mg/kg.

All mice that received 300 mg/kg died by week 2; deaths of mice that received 150 mg/kg occurred during weeks 4-8 for males and weeks 1-5 for females. Mean body weights of chemically exposed mice surviving to the end of the studies were generally 90%-94% those of vehicle controls. Sperm count and sperm motility were reduced in dosed male mice. Compound-related histopathologic lesions included demyelination of the brain in males and females that received 150 or 300 mg/kg, testicular atrophy in males at all doses, and renal tubular cell degeneration in male mice that received 300 mg/kg.

Based on reduced survival, reduced weight gain, and histopathologic lesions in the brain and kidney in rats that received 200 or 400 mg/kg and on reduced survival and histopathologic lesions of the brain in mice that received 150 or 300 mg/kg, doses selected for the 2-year studies of glycidol were 37.5 and 75 mg/kg for rats and 25 and 50 mg/kg for mice.

Body Weights and Survival in the Two-Year Studies: Mean body weights of chemically exposed male rats generally ranged from 80% to 94% of those of vehicle controls, and mean body weights of chemically exposed female rats were from 90% to 97% those of vehicle controls. Mean body weights of chemically exposed male mice were similar to those of vehicle controls; mean body weights of chemically exposed female mice were 79%-95% of those of vehicle controls. Virtually all male and female rats that received glycidol died or were killed in a moribund condition as a result of the early induction of neoplastic disease (final survival—male: vehicle control, 16/50; low dose, 0/50; high dose, 0/50; female: 28/50; 4/50; 0/50). Survival of vehicle control male rats was lower than that usually observed; however, specific causes of deaths could not be determined. The survival of male mice and low dose female mice was similar to that of vehicle controls; survival of female mice that received 50 mg/kg was lower than that of vehicle controls after week

101 (final survival—male: 33/50; 25/50; 27/50; female: 29/50; 27/50; 17/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Chemical-related nonneoplastic lesions in both rats and mice included hyperkeratosis and epithelial dysplasia of the forestomach. Fibrosis of the spleen was also present in rats of each sex, and cysts of the preputial gland and kidney were present in male mice.

Exposure to glycidol induced dose-related increases in the incidences of neoplasms in numerous tissues in both rats and mice (see summary table on page 5 of the Technical Report). In male rats, mesotheliomas arising in the tunica vaginalis and frequently metastasizing to the peritoneum were considered the major cause of early death. Early deaths in female rats were associated with the presence of mammary gland neoplasms.

Genetic Toxicology: Glycidol was mutagenic in a variety of in vitro and in vivo short-term tests. Mutagenic activity was observed in *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 exposed to glycidol with and without exogenous metabolic activation. Glycidol was positive in the absence of exogenous metabolic activation in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells; it was not tested with activation. In cytogenetic tests with CHO cells, glycidol induced both sister chromatid exchanges and chromosomal aberrations in the presence and absence of exogenous metabolic activation. Glycidol induced sex-linked recessive lethal mutations and reciprocal translocations in the germ cells of male *D. melanogaster* exposed by feeding. The incidence of micronucleated polychromatic erythrocytes was increased in the bone marrow of male $B6C3F_1$ mice administered glycidol by intraperitoneal injection.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* of glycidol for male F344/N rats, based on increased incidences of mesotheliomas of the tunica vaginalis; fibroadenomas of the mammary gland; gliomas of the brain; and neoplasms of the forestomach, intestine, skin, Zymbal gland, and thyroid gland. There was *clear evidence of carcinogenic activity* for female F344/N rats, based on increased incidences of fibroadenomas and adenocarcinomas of the mammary gland; gliomas of the brain; neoplasms of the oral mucosa, forestomach, clitoral gland, and thyroid gland; and leukemia. There was *clear evidence of carcinogenic activity* for male $B6C3F_1$ mice based on increased incidences of neoplasms of the harderian gland, forestomach, skin, liver, and lung. There was *clear evidence of carcinogenic activity* for female $B6C3F_1$ mice, based on increased incidences of neoplasms of the harderian gland, mammary gland, uterus, subcutaneous tissue, and skin. Other neoplasms that may have been related to the administration of glycidol were fibrosarcomas of the glandular stomach in female rats and carcinomas of the urinary bladder and sarcomas of the epididymis in male mice.

Synonym: 2,3-epoxy-1-propanol

Report Date: March 1990

TR-375 Toxicology and Carcinogenesis Studies of Vinyl Toluene (Mixed Isomers) (65%-71% *meta*-isomer and 32-35% *para*-isomer) (CAS No. 25013-15-4) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Vinyl Toluene is used as a monomer in the plastics and surface-coating industries. Toxicology and carcinogenesis studies were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex to vinyl toluene (mixed isomers: 65%-71% *meta* and 32%-35% *para*) by inhalation 6 hours per day, 5 days per week, for 15 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse L5178Y cells, and Chinese hamster ovary (CHO) cells.

Fifteen-Day Studies: Rats were exposed to 0, 200, 400, 800, or 1,300 ppm vinyl toluene, and mice were exposed to 0, 10, 25, 50, 100, or 200 ppm. All rats lived to the end of the studies. The mean body weights at necropsy of rats exposed to 400-1,300 ppm were 13%-19% lower than that of controls for males and 9%-13% lower for females. Most male rats exposed to 1,300 ppm had centrilobular necrosis and focal inflammatory cell infiltration of the liver, whereas minimal centrilobular vacuolization of the liver was seen in all female rats exposed to 1,300 ppm. Dysplasia of the bronchial epithelial lining, chronic bronchitis, and lymphoid hyperplasia of the lung were observed in all rats exposed to 1,300 ppm.

Three of five male mice exposed to 200 ppm vinyl toluene died before the end of the studies. Four of five male mice exposed to 200 ppm had moderate-to-severe hepatocellular necrosis; all female mice exposed to 200 ppm had hyperplasia of the epithelium of the intrapulmonary bronchi and centrilobular necrosis, vacuolization, and inflammatory cell infiltrates in the liver.

Thirteen-Week Studies: Rats were exposed to 0, 25, 60, 160, 400, or 1,000 ppm vinyl toluene. All rats lived to the end of the studies. The final mean body weights of rats exposed to 400-1,000 ppm were 8%-19% lower than that of controls for males and 6%-12% lower for females. Relative liver weights for rats at 1,000 ppm were significantly greater than those for controls. The severity of nephropathy was increased in male rats exposed to 160, 400, or 1,000 ppm. Compound-related lesions were not observed in female rats.

Mice were exposed to 0, 10, 25, 60, or 160 ppm vinyl toluene. The final mean body weights of mice exposed to 25-160 ppm were 12%-20% lower than that of controls for males and 13%-16% lower for females. Inflammation of the lung was observed in 5/10 male and 3/9 female mice exposed to 160 ppm. Metaplasia of the nasal turbinates was seen in all exposed groups.

Based on these results, 2-year studies were conducted by exposing groups of 49 or 50 rats of each sex to 0, 100, or 300 ppm vinyl toluene by inhalation, 6 hours per day, 5 days per week for 103 weeks. Groups of 50 mice of each sex were exposed to 0, 10, or 25 ppm on the same schedule.

Body Weights and Survival in the Two-Year Studies: Mean body weights of male rats exposed to 300 ppm vinyl toluene and those of female rats exposed to 100 and 300 ppm were generally 4%-11% lower than those of controls. No significant differences in survival were seen between any groups of rats of either sex (male: control, 19/49; low dose, 17/50; high dose, 19/50; female: 31/50; 28/50; 26/50). Mean body weights of mice exposed to 25 ppm were 10%-23% lower than those of controls after week 8, whereas mice exposed to 10 ppm showed a weight decrement that was generally less than 10%. The survival of male mice exposed to 25 ppm was significantly greater than that of controls. No other significant differences in survival were seen between any groups of mice of either sex (male: 33/50; 30/50; 41/50; female: 36/50; 37/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Degenerative and nonneoplastic proliferative lesions of the nasal mucosa were observed at increased incidences in exposed rats. These lesions included diffuse hyperplasia (goblet cell) of the respiratory epithelium with intraepithelial mucous cysts and focal erosion of the olfactory epithelium with cystic dilation (cysts) of the Bowman's glands. Focal respiratory epithelial metaplasia of the olfactory epithelium was seen in some exposed males, and cells with homogeneous eosinophilic cytoplasm in the olfactory epithelium occurred at increased incidences in exposed female rats. Neoplasms of the nasal mucosa were not seen in male or female rats.

There were no chemically related increases in neoplasm incidence in exposed male or female rats.

Degenerative and inflammatory lesions of the nasal mucosa were observed at increased incidences in exposed mice. These lesions included focal chronic active inflammation and diffuse hyperplasia of the respiratory epithelium. Chronic active inflammation of the bronchioles occurred in many exposed mice but not in controls. Neoplasms of the nasal passage were not observed in mice.

There were no chemically related increases in neoplasm incidence in exposed male or female mice. Exposure-related decreased incidences included alveolar/bronchiolar neoplasms (control, 12/50; 10 ppm, 5/49; 25 ppm, 2/49) and malignant lymphomas (7/50; 3/50; 0/50) in males and hepatocellular neoplasms (9/48; 5/16; 2/49) in females.

Genetic Toxicology: Vinyl toluene did not induce gene mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation (S9). Vinyl toluene was positive in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y/TK cells in the absence of S9; it was not tested with S9. Vinyl toluene did not induce sister chromatid exchanges or chromosomal aberrations in CHO cells with or without S9.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* for male or female F344/N rats exposed to 100 or 300 ppm vinyl toluene and *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice exposed to 10 or 25 ppm.

There was evidence of chemical-related toxicity to the nasal passage in both rats and mice.

Synonyms: 3-vinyl toluene and 4-vinyl toluene (mixed isomers)

Report Date: March 1990

TR-376 Toxicology and Carcinogenesis Studies of Allyl Glycidyl Ether (CAS No. 106-92-3) in Osborne-Mendel Rats and $B6C3F_1$ Mice (Inhalation Studies)

Allyl glycidyl ether is used as a resin intermediate and as a stabilizer of chlorinated compounds, vinyl resins, and rubber. Toxicology and carcinogenesis studies were conducted by exposing groups of Osborne-Mendel rats and $B6C3F_1$ of each sex to allyl gylcidyl ether (greater than 97% pure) by inhalation for 6 hours per day, 5 days per week for 2 weeks, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*. Studies of reproductive effects were conducted in rats and mice exposed to allyl glycidyl ether for 8 weeks.

Two-Week Studies: Exposure concentrations ranged up to 500 ppm in rats and 100 ppm in mice. All rats that were exposed to 500 ppm died; no deaths occurred at the next lower (200 ppm) exposure concentration. All male mice and 3/5 female mice exposed to 100 ppm and 2/5 male mice and 1/5 female mice exposed to 50 ppm died. Compound-related lesions in rats and mice included acute inflammation of the nasal passage and major airways.

Eight-Week Studies of Reproductive Effects: Rats were exposed to 0-200 ppm allyl glycidyl ether, and mice were exposed to 0-30 ppm, 6 hours per day, 5 days per week for 8 weeks. The mating performance of exposed male rats was markedly reduced; however, sperm motility and number were not affected. No deficiencies were seen in the reproductive performance of exposed female rats or male or female mice.

Thirteen-Week Studies: Exposure concentrations ranged up to 200 ppm for rats and 30 ppm for mice. All rats lived to the end of the studies. The final mean body weights of male rats exposed to 10-200 ppm were 7%-24% lower than that of controls. Clinical signs attributable to irritation of the upper respiratory tract and eyes were seen in exposed animals. Histologic lesions included squamous metaplasia of the nasal passage in all exposure groups (4 ppm, lowest concentration) and involved both the respiratory epithelium and the olfactory epithelium. The lesions were more severe anteriorly and dorsally and with increasing concentration. At 30 ppm and higher, erosion was seen in the nasal passage and squamous metaplasia was seen in the upper airways.

There were no compound-related deaths in mice. The final mean body weights of mice exposed to 30 ppm were 12% lower than those of controls for both males and females. Mice exposed to 10 or 30 ppm allyl glycidyl ether

had squamous metaplasia of the nasal passage, involving both the respiratory epithelium and the olfactory epithelium, which tended to be more severe in the anterior and dorsal portions of the nasal passage. In mice exposed to 30 ppm, epithelial erosions were also found.

Body Weights and Survival in the Two-Year Studies: Two-year studies were conducted by exposing groups of 50 Osborne-Mendel rats and $B6C3F_1$ mice of each sex to 0, 5, or 10 ppm allyl glycidyl ether by inhalation for 6 hours per day, 5 days per week for 102 or 103 weeks. Mean body weights of the exposed rats were within 8% of those of controls throughout the studies. Mean body weights of mice exposed to 5 or 10 ppm were 5%-20% lower than those of controls. Deaths were seen in all groups of male rats beginning at 1 year of age (final survival – control, 12/50; 5 ppm, 11/50; 10 ppm, 8/50). Survival of female rats was not exposure related (24/50; 30/50; 25/50). Exposed mice had slightly increased survival (male mice: 38/50; 39/50; 46/50; female mice: 33/50; 42/50; 41/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: In male rats exposed to 10 ppm allyl glycidyl ether, three apparently unrelated neoplasms of the nasal passage were found. Two neoplasms, a papillary adenoma and a squamous cell carcinoma, appeared to arise from different cell types in the respiratory epithelium. One poorly differentiated adenocarcinoma in the olfactory region was also found. One papillary adenoma of respiratory epithelial origin was found in a female rat exposed to 5 ppm. Exposure-related nonneoplastic lesions of the nasal passages in rats included inflammation, squamous metaplasia, respiratory metaplasia (replacement of olfactory epithelium by ciliated epithelium), hyperplasia of the respiratory epithelium, and degeneration of the olfactory epithelium. In male mice exposed to 10 ppm allyl glycidyl ether, a hemangioma and three papillary adenomas were present in the nasal passage. In female mice exposed to 10 ppm, a hemangioma and an adenoma were found in the nasal passage. Nonneoplastic lesions of the nasal passages in mice included inflammation, squamous metaplasia, hyperplasia, basal cell hyperplasia, dysplasia of the respiratory epithelium, and metaplasia of the olfactory epithelium. In male mice, there was an exposure-related decrease in the incidences of hepatocellular neoplasms; in female mice, there was a decrease in the incidences of pituitary gland adenomas.

Genetic Toxicology: Allyl glycidyl ether was mutagenic in *S. typhimurium* strains TA100 and TA1535 with and without exogenous metabolic activation; no mutagenic activity was observed in strains TA98 or TA1537. Allyl glycidyl ether induced sister chromatid exchanges and chromosomal aberrations in CHO cells both in the presence and the absence of metabolic activation. A significant increase in sex-linked recessive lethal mutations was recorded in the germ cells of male *D. melanogaster* fed a sucrose solution containing allyl glycidyl ether, but no increase in reciprocal translocations occurred in these cells.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *equivocal evidence of carcinogenic activity* of allyl glycidyl ether for male Osborne-Mendel rats, based on the presence of one papillary adenoma of respiratory epithelial origin, one squamous cell carcinoma of respiratory epithelial origin, and one poorly differentiated adenocarcinoma of olfactory epithelial origin, all occurring in the nasal passage of males exposed to 10 ppm. There was *no evidence of carcinogenic activity* of allyl glycidyl ether for female rats. One papillary adenoma of the respiratory epithelium was present in a female rat exposed to 5 ppm. There was *some evidence of carcinogenic activity* of allyl glycidyl ether for male $B6C3F_1$ mice, based on the presence of three adenomas of the respiratory epithelium, dysplasia in four males, and focal basal cell hyperplasia of the respiratory epithelium in seven males in the nasal passage of mice exposed to 10 ppm. There was *equivocal evidence of carcinogenic activity* of allyl glycidyl ether for female mice, based on the presence of one adenoma of the respiratory epithelium and focal basal cell hyperplasia of the respiratory epithelium in seven females exposed to 10 ppm. The sensitivity of the assay to detect potential carcinogenicity may have been reduced in male rats because of poor survival in all groups.

In exposed mice, body weights were decreased 10% or more, mortality was decreased, and there were lower incidences of liver neoplasms (males) and pituitary gland adenomas (females) compared with controls.

Significant exposure-related nonneoplastic lesions were restricted to the nasal passage in both rats and mice and induced inflammation, metaplasia, respiratory epithelial hyperplasia, and olfactory epithelial degeneration. Basal cell hyperplasia and dysplasia of the respiratory epithelium of the nasal passage were found only in the mice.

Synonyms: allyl 2,3-epoxypropyl ether; 1-allyloxy-2,3-epoxypropane; 1,2-epoxy-3-allyloxypropane; glycidyl allyl ether; ((2-propenyloxy)methyl)oxirane; 1-(allyloxy)-2,3-epoxypropane

Report Date: January 1990

TR-377 Toxicology and Carcinogenesis Studies of CS2 (94% *o*-Chlorobenzalmalononitrile, CAS No. 2698-41-1) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

CS2 (94% *o*-chlorobenzalmalononitrile [CS]; 5% Cab-O-Sil® colloidal silica; 1% hexamethyldisilizane), an eye and respiratory irritant, is used as an aerosol to control riots. Toxicology and carcinogenesis studies were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex for 6 hours per day, 5 days per week for 2 weeks, 13 weeks, or 2 years, to a CS2 aerosol. Genetic toxicology studies with CS2 were conducted in *Salmonella typhimurium*, mouse lymphoma cells, and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: At exposure concentrations of 0, 1, 3, 10, 30, or 100 mg/m^3 CS2, all rats exposed to 30 or

100 mg/m³ and all mice exposed to 10, 30, or 100 mg/m³ died before the end of the studies. Compound-related clinical signs observed included erythema, blepharospasm, listlessness, nasal discharge, and mouse breathing.

Thirteen-Week Studies: At exposure concentrations of 0, 0.4, 0.75, 1.5, 3, or 6 mg/m³, 1/10 male rats exposed to 6 mg/m³ died before the end of the studies. Final mean body weights of rats exposed to 1.5 mg/m³ or more were 17%-44% lower than that of controls for males and 10%-24% lower for females. The absolute and relative thymus weights were reduced for exposed male and female rats, particularly at 6 mg/m³. Compound-related lesions of the nasal passage in rats included focal erosion with regenerative hyperplasia and squamous metaplasia of the respiratory epithelium and suppurative inflammation. Acute inflammation and hyperplasia of the respiratory epithelium were seen in the larynx and trachea of some exposed rats.

All mice exposed to 6 mg/m³ and 1/10 males and 1/10 females exposed to 3 mg/m³ died before the end of the studies. Final mean body weights of mice exposed to 3 mg/m³ were 13% lower than that of controls for males and 9% lower for females. Compound-related lesions of the nasal passage in mice included squamous metaplasia of the nasal respiratory epithelium and inflammation.

Based on these results, CS2 exposure concentrations for the 2-year studies were 0, 0.075, 0.25, or 0.75 mg/m³ for 6 hours per day, 5 days per week for 105 weeks for groups of 50 rats of each sex. Groups of 50 mice of each sex were exposed to 0, 0.75, or 1.5 mg/m³ on the same schedule.

Body Weights and Survival in the Two-Year Studies: Final mean body weights of rats exposed to 0.75 mg/m³ were 7%-11% lower than those of controls. Final mean body weights of mice exposed to CS2 were lower than those of controls (male: 5% and 9%; female: 10% and 17%). No compound-related clinical signs were observed. No significant differences in survival were seen for any group of rats or mice of either sex.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Compound-related nonneoplastic lesions occurred in the nasal passage of exposed rats and mice. In exposed rats, hyperplasia and squamous metaplasia of the respiratory epithelium and degeneration of the olfactory epithelium with ciliated columnar and/or squamous metaplasia were observed. Focal chronic inflammation and proliferation of the periosteum of the turbinate bones were increased slightly in rats at the top exposure concentration. Suppurative inflammation with hyperplasia and squamous metaplasia of the respiratory epithelium occurred in exposed mice.

There were no compound-related increased incidences of neoplasms in rats or mice exposed to CS2. In exposed female mice, there were pronounced decreases in the incidences of adenomas of the pituitary pars distalis (control, 13/47; 0.75 mg/m³, 5/46; 1.5 mg/m³, 1/46) and decreased incidences of malignant lymphomas (21/50; 12/50; 8/50).

Genetic Toxicology: The responses in Salmonella gene mutation tests with CS2 were equivocal in one laboratory for strain TA100 in the absence of exogenous metabolic activation (S9) and equivocal in another laboratory for TA97 with S9; in all other strains tested, CS2 was clearly negative with or without S9. CS2 induced trifluorothymidine resistance in mouse L5178Y/TK lymphoma cells in the absence of S9; it was not tested with S9. CS2 induced both sister chromatid exchanges and chromosomal aberrations in CHO cells with and without S9.

Conclusions: Under the conditions of these inhalation studies, there was *no evidence of carcinogenic activity* of CS2 for male or female F344/N rats exposed to 0.075, 0.25, or 0.75 mg/m³ in air for up to 2 years. There was *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice exposed to 0.75 or 1.5 mg/m³ in air for up to 2 years. Concentration-related decreases in the incidences of pituitary gland adenomas and lymphomas were observed in female mice.

Exposure to CS2 caused degeneration and squamous metaplasia of the olfactory epithelium, hyperplasia and metaplasia of the respiratory epithelium, and proliferation of the periosteum of the nasal passage of rats. In mice, exposure to this compound caused suppurative inflammation and hyperplasia and squamous metaplasia of the respiratory epithelium of the nasal passage.

Report Date: March 1990

TR-378 Toxicology and Carcinogenesis Studies of Benzaldehyde (CAS No. 100-52-7) in F344/N Rats and $B6C3F_1$ Mice (Gavage Studies)

Benzaldehyde is an aromatic aldehyde used in the food, beverage, pharmaceutical, perfume, soap, and dyestuff industries. Toxicology and carcinogenesis studies were conducted by administering benzaldehyde (99% pure) in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

Sixteen-Day Studies: All rats that received 1,600 mg/kg died by day 2, and 2/5 males and 2/5 females that received 800 mg/kg died before the end of the studies. Final mean body weights of dosed and vehicle control rats were similar, with the exception of the 800 mg/kg groups, in which males were 14% lighter and females were 11% lighter than vehicle controls. All mice that received 1,600 or 3,200 mg/kg died by day 3. Final mean body weights of dosed and vehicle control mice were similar. No gross lesions attributable to benzaldehyde were detected upon necropsy.

Thirteen-Week Studies: Six of 10 male rats and 3/10 female rats that received 800 mg/kg and 1/10 female rats that received 400 mg/kg died near the end of the studies. Final mean body weights of dosed and vehicle control

rats were similar, with the exception of male rats receiving 800 mg/kg, which were 26% lighter than vehicle controls. Compound-related lesions seen in rats receiving 800 mg/kg, but not in those receiving 400 mg/kg, included degeneration and necrosis in the cerebellum, necrosis in the hippocampus, hyperplasia and/or hyperkeratosis in the forestomach, and degeneration or necrosis of the liver and of the tubular epithelium in the kidney.

Nine of 10 male mice and 1/10 female mice that received 1,200 mg/kg benzaldehyde died by the end of the first week. Compound-related renal tubule degeneration and/or necrosis and reduction in final body weight were observed in the 600 mg/kg group of male mice. No reductions in body weight or compound-related lesions were seen in female mice.

Based on observations of compound-related lesions involving the brain, forestomach, kidney, and liver of male and female rats and the kidney of male mice in the 13-week studies, 2-year studies were conducted by administering 0, 200, or 400 mg/kg benzaldehyde in corn oil by gavage, 5 days per week for 103 weeks to groups of 50 male and 50 female rats and for 104 weeks to groups of 50 male mice. Based on survival data from the 16-day and 13-week studies, groups of 50 female mice were administered 0, 300, or 600 mg/kg benzaldehyde for 103 weeks.

Body Weights and Survival in the Two-Year Studies: Mean body weights of dosed rats and mice were similar to their respective vehicle controls throughout the studies. The survival of the high dose group of male rats was lower than that of the vehicle controls after 1 year; no other significant differences were observed between any groups of rats or mice (survival—male rats; vehicle control, 37/50; low dose, 29/50; high dose, 21/50; female rats: 33/50; 33/50; 29/50; male mice: 32/50; 33/50; 31/50, female mice: 30/50; 27/50; 35/50).

Nonneoplastic and Neoplastic Effects in the Two-year Studies: The only effects of benzaldehyde were those seen in the forestomach of mice. The incidences of uncommonly occurring squamous cell papillomas of the forestomach in both exposure groups were significantly greater than those in vehicle controls (male: vehicle control, 1/50; low dose, 2/50; high dose, 5/50; female: 0/50; 5/50; 6/50). The increased incidences of papillomas were accompanied by dose-related increases in the incidences in forestomach hyperplasia (male: 7/50; 8/50; 16/50; female: 12/50; 23/50; 39/50).

Genetic Toxicology: Benzaldehyde was not mutagenic in six strains of *S. typhimurium* and did not induce chromosomal aberrations in CHO cells, with or without exogenous metabolic activation. Benzaldehyde induced increases in trifluorothymidine-resistant mouse lymphoma cells in the absence exogenous metabolic activation and increased sister chromatid exchanges in CHO cells in both the presence and absence of metabolic activation. Sex-linked recessive lethal mutations were not induced in the germ cells of adult male *D. melanogaster* administered benzaldehyde by feeding or by injection.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of benzaldehyde for male or female F344/N rats receiving 200 or 400 mg/kg per day. There was *some evidence of carcinogenic activity* of benzaldehyde for male or female $B6C3F_1$ mice, as indicated by increased incidences of squamous cell papillomas and hyperplasia of the forestomach. Female rats and male and female mice might have been able to tolerate higher doses.

Synonyms: artificial almond oil; artificial essential oil of almond; benzenecarbonal; benzene carbaldehyde; benzoic aldehyde; phenylmethanal

Report Date: March 1990

TR-379 Toxicology and Carcinogenesis Studies of 2-Chloroacetophenone (CAS No. 532-27-4) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

2-Chloroacetophenone is a potent lacrimator that has been used as a riot control agent and in tear gas formulations for personal protection devices. Toxicology and carcinogenesis studies were conducted by exposing groups of F344/N rats and $B6C3F_1$ mice of each sex to air containing 2-chloroacetophenone vapor for 14 days, 13 weeks, 15 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: In 14-day studies, exposure concentrations of 2-chloroacetophenone ranged from 4.8 to 64 mg/m^3. All rats exposed to 19, 43, or 64 mg/m^3 died during the first week of the studies and 1/5 male rats exposed to 10 mg/m^3 died during the second week of the study. Rats exposed to 10 mg/m^3 lost weight; the final mean body weights of male or female rats exposed to 4.8 mg/m^3, the lowest concentration used, were 23% or 15% lower than that of controls. During the exposure, rats showed partial closure of the eyelids, excessive lacrimation (dacryorrhea), dyspnea, and erythema. All mice exposed to 10 mg/m^3 or higher concentrations of 2-chloroacetophenone died during the first week of the studies. The final mean body weights of mice exposed to 4.8 mg/m^3 were similar to those of controls. Dacryorrhea was observed in exposed mice.

Thirteen-Week Studies: The exposure concentrations of 2-chloroacetophenone ranged from 0.25 to 4 mg/m^3 for rats and mice. All rats lived to the end of the studies. The final mean body weights of rats exposed to 4 mg/m^3 were 9% lower than those of controls. Eye irritation during exposure was evident in rats exposed to 0.5 mg/m^3 or higher concentrations of 2-chloroacetophenone. One of 10 female mice exposed to 4 mg/m^3 and 1/10 female mice exposed to 0.5 mg/m^3 died before the end of the study. The final mean body weights of exposed mice were 7%-12% lower than that of controls for males and 12%-15% lower for females. No chemical-related gross or microscopic lesions were observed in rats or mice.

In the 2-year studies, groups of 60 rats of each sex were exposed to a vapor of 0 (chamber control), 1, or 2 mg/m^3 (0, 0.15, or 0.3 ppm) 2-chloroacetophenone, 6 hours per

day, 5 days per week. Groups of 60 mice of each sex were exposed to 0 (chamber control), 2, or 4 mg/m^3 (0, 0.3, or 0.6 ppm) on the same schedule. Ten animals from each group were killed and examined at 15 months; the remaining animals continued on study for 2 years.

Fifteen-Month Studies: In the 15-month studies, minimal-to-mild focal squamous metaplasia and hyperplasia of the respiratory epithelium were seen at increased incidences in rats exposed to 2 mg/m^3. No exposure-related lesions were observed in mice of either sex.

Body Weight and Survival in the Two-Year Studies: Mean body weights and survival of exposed and chamber control rats were similar throughout most of the studies (survival-male: control, 14/50; 1 mg/m^3, 22/50; 2 mg/m^3, 17/50; female: 23/50; 20/50; 24/50). Mean body weights of male mice exposed to 4 mg/m^3 were about 5%-12% lower than those of controls after week 30; small differences between mean body weights of exposed and control female mice were not clearly exposure related. The survival of female mice exposed to 2 mg/m^3 was significantly lower than that of chamber controls after week 98. No other differences in survival were observed between any groups of mice (male: control, 34/50; 2 mg/m^3, 36/50; 4 mg/m^3, 33/50; female: 40/50; 28/50; 32/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Fibroadenomas of the mammary gland occurred in female rats with positive trends, and the incidence in the 2 mg/m^3 group was greater than that in chamber controls (control, 12/50; 1 mg/m^3, 19/50; 2 mg/m^3, 23/50). The incidences of adenomas or adenocarcinomas of the mammary gland were not increased in the exposed groups.

Minimal-to-mild suppurative inflammation of the nasal mucosa was observed at increased incidences in exposed male rats. Hyperplasia and squamous metaplasia of the nasal respiratory epithelium were observed at increased incidences in exposed male and female rats. In mice, squamous metaplasia of the respiratory epithelium of the nasal passage was seen in four females and two males exposed to 4 mg/m^3 2-chloroacetophenone.

Inflammation, ulcers, and squamous hyperplasia of the forestomach were observed at increased incidences in exposed female rats.

Genetic Toxicology: 2-Chloroacetophenone was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation. In cytogenetic tests with CHO cells, 2-chloroacetophenone did not induce sister chromatid exchanges with or without activation, but a weak positive increase in chromosomal aberrations was observed in the absence of metabolic activation.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* of 2-chloroacetophenone for male rats exposed to 1 or 2 mg/m^3. There was *equivocal evidence of carcinogenic activity* for female F344/N rats, based on a marginal increase in fibroadenomas of the mammary gland. There was *no evidence of carcinogenic activity* for male or female B6C3F$_1$ mice exposed to 2 or 4 mg/m^3 2-chloroacetophenone.

Synonyms: α-chloroacetophenone; 2-chloro-1-phenylethanone; CN; phenacyl chloride; phenylchloromethylketone

Trade Names: Mace®; Chemical Mace®

Report Date: March 1990

TR-380 Toxicology and Carcinogenesis Studies of *l*-Epinephrine Hydrochloride (CAS No. 55-31-2) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies)

l-Epinephrine, an endogenous neurotransmitter hormone, is widely used for the treatment of allergic and respiratory disorders. Toxicology and carcinogenesis studies of epinephrine hydrochloride were conducted by exposing groups of F344/N rats and B6C3F$_1$ mice of each sex to an aerosol containing epinephrine hydrochloride for 14 days, 13 weeks, 15 months, or 2 years. During the 14-day and 13-week studies, control animals were exposed to dilute aerosols of hydrochloric acid (pH 2.8), whereas during the 15-month and 2-year studies, controls were exposed to aerosols of water. Genetic toxicology studies of epinephrine were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: Rats and mice were exposed to 0 or 12.5-200 mg/m^3 epinephrine hydrochloride. Deaths occurred in male rats exposed to 12.5 mg/m^3 or more and in females exposed to 25 mg/m^3 or more. Deaths of mice occurred at concentrations of 50 mg/m^3 or higher. Compound-related clinical signs included an increased respiratory rate in all groups of epinephrine-exposed rats and mice. At higher concentrations (100 and 200 mg/m^3), excessive lacrimation and dyspnea in rats and exaggerated visual and auditory reflexes in mice were observed.

Thirteen-Week Studies: Rats and mice were exposed to 0 or 2.5-40 mg/m^3 epinephrine hydrochloride. Deaths in rats and mice were not concentration related. Final mean body weights of chemically exposed and hydrochloric acid aerosol control rats and mice were generally similar. Increased respiratory rates were noted in rats and mice exposed to 40 mg/m^3. Heart and adrenal gland weights of rats and mice and liver weights of mice exposed to 40 mg/m^3 were greater than those of aerosol controls. Squamous metaplasia occurred in the respiratory epithelium of the nasal mucosa of rats and mice exposed to 40 mg/m^3. Degenerative lesions of the laryngeal muscle were seen in male and female rats exposed to 20 or 40 mg/m^3. Inflammation in the glandular stomach was seen in male and female mice exposed to 10, 20, and 40 mg/m^3, and uterine atrophy was seen in 7/10 female mice exposed to 40 mg/m^3.

Two-year studies were conducted by exposing groups of 60 rats or each sex to 0, 1.5, or 5 mg/m^3 epinephrine hydrochloride, 5 days per week for 103 weeks. Groups of 60 mice of each sex were exposed to 0, 1.5, or 3 mg/m^3

epinephrine hydrochloride, 5 days per week for 104 weeks. Use of these exposure concentrations represented a departure from the usual practice of utilizing doses equivalent to one-half the maximum tolerated dose (MTD) and the MTD for 2-year carcinogenicity studies. Thus, although the dose levels exceeded maximum human therapeutic use levels (normalized to body weight and surface area), they were less than one-half the MTD.

Fifteen-Month Studies: Results of hematologic analyses did not show compound-related changes. Absolute liver weights for exposed mice (3 mg/m^3) and rats (5 mg/m^3) and relative liver weights for exposed rats (5 mg/m^3) were significantly lower than those for controls. The absolute kidney weights for mice exposed to 3 mg/m^3 and the kidney weight to body weight ratio for male mice exposed to 3 mg/m^3 were significantly lower than those for controls. No compound-related lesions were seen in rats or mice.

Body Weights and Survival in the Two-Year Studies: Mean body weights and survival of exposed and control rats and mice were similar (survival, rats – male: control, 33/50; 1.5 mg/m^3, 27/50; 5 mg/m^3, 32/50; female: 32/50; 29/50; 30/50; mice – male: control, 33/50; 1.5 mg/m^3, 34/50; 3 mg/m^3, 36/50; female: 32/50; 35/50; 34/50).

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Suppurative inflammation of the nasal mucosa, dilatation of the nasal glands (Bowman's and septal), and hyperplasia of the respiratory epithelium were seen at increased incidences in male rats exposed to 5 mg/m^3 and in female rats exposed to 1.5 or 5 mg/m^3.

Hyaline degeneration of the olfactory epithelium in male mice and suppurative inflammation of the nasal passage and hyaline degeneration of the respiratory epithelium in female mice were increased in the 1.5 and 3 mg/m^3 groups compared with controls. No neoplasms seen in these studies were considered related to chemical exposure.

Genetic Toxicology: Salmonella gene mutation tests with *l*-epinephrine yielded negative results in strain TA100 in the presence of exogenous metabolic activation (S9) and equivocal results in the absence of S9. No mutagenic activity was observed in strains TA98, TA1535, or TA1537 with or without S9. The responses observed in the CHO cell assay for induction of sister chromatid exchanges were considered to be negative and equivocal in the presence and absence of S9 activation, respectively. *l*-Epinephrine did not induce chromosomal aberrations in CHO cells with or without S9.

Conclusions: Under the conditions of these 2-year studies, no carcinogenic effects were observed in male or female F344/N rats exposed to aerosols containing 1.5 or 5 mg/m^3 *l*-epinephrine hydrochloride for 2 years or in B6C3F$_1$ mice exposed to 1.5 or 3 mg/m^3 *l*-epinephrine hydrochloride for 2 years. However, these studies were considered to be *inadequate studies of carcinogenic activity* because the concentrations used, which were chosen to represent multiples of therapeutic doses, were considered too low for the animals to have received an adequate systemic challenge from the compound.

Synonyms: adrenaline hydrochloride; 4-(1-hydroxy-2-(methylamino)ethyl)-1,2-benzenediol; (-)3,4-dihydroxy-α-((methylamino)methyl)benzyl alcohol hydrochloride; methylaminoethanol catechol hydrochloride

Trade names for epinephrine formulations: Primatene® Mist; Sus-Phrine®; Epipen®; Supravenin Hydrochloride®; Bronkaid®

Report Date: March 1990

TR-381 Toxicology and Carcinogenesis Studies of *d*-Carvone (CAS No. 2244-16-8) in B6C3F$_1$ Mice (Gavage Studies)

d-Carvone occurs naturally in caraway and dill seeds and in many essential oils; it has been used as a carminative and in perfumes and soaps. Toxicity and carcinogenesis studies were conducted by administering *d*-carvone (approximately 96% pure) in corn oil by gavage to groups of male and female B6C3F$_1$ mice for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Sixteen-Day Studies: All mice that received 1,600 or 3,500 mg/kg died within 7 days. Relative liver weights were increased for dosed male mice, and relative thymus weights were decreased for dosed female mice. No compound-related lesions were observed.

Thirteen-Week Studies: All male mice and 9/10 female mice that received the top dose of 1,500 mg/kg died before the end of the studies. No compound-related histopathologic changes were observed.

Based on survival at the high doses in the 13-week studies, 2-year toxicology and carcinogenesis studies were conducted by administering *d*-carvone in corn oil by gavage to groups of 50 male and 50 female mice at doses of 375 or 750 mg/kg, 5 days per week for 103 weeks.

Two-Year Studies: Mean body weights of dosed and vehicle control mice were similar throughout the studies. Survival of dosed male mice was similar to that of vehicle controls (vehicle control, 37/50; low dose, 42/50; high dose, 36/50); survival of dosed female mice was greater than that of vehicle control female mice (14/50; 29/50; 38/50). Apparently, abscesses in the urogenital system caused the early deaths of many vehicle control female mice.

No neoplastic lesions attributed to *d*-carvone dosing were observed in mice.

Genetic Toxicology: *d*-Carvone was not mutagenic in *S. typhimurium* but induced sister chromatid exchanges and chromosomal aberrations in CHO cells.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of *d*-carvone for male or female B6C3F$_1$ mice administered 375 or 750 mg/kg, 5 days per week for 2 years.

Synonyms for *d*-carvone: (+)-carvone; *d*(+)-carvone; (*S*)-carvone; (*S*)-(+)carvone; (*S*)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one; (*S*)-*d*-*p*-mentha-6-8,(9)-dien-2-one; (*S*)-(+)-*p*-mentha-6,8-dien-2-one; *d*-1-methyl-4-isopropenyl-6-cyclohexen-2-one. Carvol is a synonym for carvone (*d*, *l* not specified)

Report Date: February 1990

TR-382 Toxicology and Carcinogenesis Studies of Furfural (CAS No. 98-01-1) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Furfural is used as a precursor for the manufacture of furan, furfuryl alcohol, tetrahydrofuran, and their derivatives and as an industrial solvent. Furfural is also present in numerous processed food and beverage products. Toxicology and carcinogenesis studies were conducted by administering furfural (99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, Chinese hamster ovary (CHO) cells, *Drosophila melanogaster*, and mouse bone marrow cells.

Sixteen-Day Studies: Rats received doses ranging from 15 to 240 mg/kg, and mice received doses from 25 to 400 mg/kg. Eight of 10 rats that received 240 mg/kg died within 3 days. Final mean body weights of chemically exposed animals were similar to those of vehicle controls; no compound-related histologic lesions were observed in any dosed groups.

Thirteen-Week Studies: Rats received doses ranging from 11 to 180 mg/kg, and mice received doses from 75 to 1,200 mg/kg. Most rats that received 180 mg/kg died; mean body weights of chemically exposed rats were similar to those of vehicle controls. Mean relative and absolute liver and kidney weights were increased in male rats that received 90 mg/kg, and cytoplasmic vacuolization of hepatocytes was increased in chemically exposed male rats.

Almost all mice that received doses of 600 or 1,200 mg/kg died within the first 3 weeks. Mean body weights of chemically exposed mice were similar to those of vehicle controls throughout the studies. Mean absolute liver weights and liver weight to body weight ratios were increased in females that received 300 mg/kg. Centrilobular coagulative necrosis and/or multifocal subchronic inflammation of the liver were present in chemically exposed mice but not in vehicle control mice.

Based on these results, doses selected for the 2-year studies were 0, 30, and 60 mg/kg for rats and 0, 50, 100, and 175 mg/kg for mice.

Body Weight and Survival in the Two-Year Studies: Mean body weights of chemically exposed and vehicle control animals were similar throughout the studies for rats and mice. Two-year survival of male rats; low dose female rats, and mice was unaffected by chemical exposure (male rats: vehicle control, 31/50; low dose, 28/50; high dose, 24/50; female rats: 28/50; 32/50; 18/50; male mice: vehicle control, 35/50; low dose, 28/50; mid dose, 24/50; high dose, 27/50; female mice: 33/50; 28/50; 29/50; 32/50). Survival of high dose female rats was reduced by deaths associated with gavage administration; the administration of furfural was considered to be a contributing factor in these gavage-related deaths.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: Centrilobular necrosis of the liver occurred at increased incidences in chemically exposed male rats (vehicle control, 3/50; low dose, 9/50; high dose, 12/50). Two high dose male rats had bile duct dysplasia with fibrosis, and two had cholangiocarcinomas; neither lesion was seen in the other dose groups. The historical incidence of bile duct neoplasms in corn oil vehicle control male rats is 3/2,145 (0.1%).

Multifocal pigmentation and chronic inflammation of the subserosa of the liver occurred in chemically exposed mice (pigmentation – male: 0/50; 0/50; 8/49; 18/50; female: 0/50; 0/50; 0/50; 11/50; chronic inflammation – male: 0/50; 0/50; 8/49; 18/50; female: 0/50; 0/50; 1/50; 8/50). The incidences of hepatocellular adenomas and hepatocellular carcinomas in male mice and hepatocellular adenomas in female mice were significantly increased in the high dose group compared with those in the vehicle controls (male – adenomas: 9/50; 13/50; 11/49; 19/50; carcinomas: 7/50; 12/50; 6/49; 21/50; female – adenomas: 1/50; 3/50; 5/50; 8/50; adenomas or carcinomas, combined: 5/50; 3/50; 7/50; 12/50).

Three renal cortical adenomas or carcinomas occurred in chemically exposed male mice (0/50; 1/50; 1/49; 1/50), and a renal cortical adenoma was present in one low dose female mouse; the historical incidence of renal cortical neoplasms in National Toxicology Program 2-year corn oil gavage studies in male B6C3F$_1$ mice is 8/2,183.

Forestomach hyperplasia occurred in chemically exposed female mice, and squamous cell papillomas were increased in high dose female mice (hyperplasia: 0/50; 5/50; 5/50; 3/50; papillomas: 1/50; 0/50; 1/50; 6/50).

Genetic Toxicology: In gene mutation tests with four strains of Salmonella (TA98, TA100, TA1535, and TA1537), no mutagenic activity was observed in the presence or absence of exogenous metabolic activation (S9) in one laboratory and an equivocal response was observed in TA100 in the absence of S9 in a second laboratory. Exposure to furfural induced trifluorothymidine resistance in mouse L5178Y lymphoma cells in the absence of S9 (no evaluation was made in the presence of S9), sister chromatid exchanges (SCEs) and chromosomal aberrations in CHO cells in the presence or absence of S9, and an increase in sex-linked recessive lethal mutations but no reciprocal translocations in germ cells of *D. melanogaster*; furfural did not induce SCEs or chromosomal aberrations in the bone marrow of B6C3F$_1$ mice.

Conclusions: Under the conditions of these 2-year gavage studies, there was *some evidence of carcinogenic activity* of furfural for male F344/N rats based on

the occurrence of uncommon cholangiocarcinomas in two animals and bile duct dysplasia with fibrosis in two other animals. There was *no evidence of carcinogenic activity* for female F344/N rats that received doses of 0, 30, or 60 mg/kg furfural. There was *clear evidence of carcinogenic activity* for male B6C3F$_1$ mice, based on increased incidences of hepatocellular adenomas and hepatocellular carcinomas. There was *some evidence of carcinogenic activity* in female B6C3F$_1$ mice, based on increased incidences of hepatocellular adenomas. Renal cortical adenomas or carcinomas in male mice and squamous cell papillomas of the forestomach in female mice may have been related to exposure to furfural.

Synonyms: 2-furancarboxaldehyde; 2-furaldehyde; pyromucic aldehyde

Common Name: Artificial oil of ants

Report Date: March 1990

TR-383 Toxicology and Carcinogenesis Studies of 1-Amino-2,4-Dibromoanthraquinone (CAS: 81-49-2) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-384 Toxicology and Carcinogenesis Studies of 1,2,3-Trichloropropane (CAS No. 96-18-4) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-385 Toxicology and Carcinogenesis Studies of Methyl Bromide (CAS: 74-83-9) in B6C3F$_1$ Mice (Inhalation Studies)

Methyl bromide is widely used as a fumigant and pesticide. Toxicology and carcinogenesis studies were conducted by exposing groups of male and female B6C3F$_1$ mice to methyl bromide (99.8% pure) by inhalation 6 hours per day, 5 days per week, for 14 days, 6 weeks, 13 weeks, or 2 years. Six-week and 13-week inhalation toxicity studies in F344/N rats were conducted concurrently with the mouse studies. Hematology parameters were measured during the 6-week, 13-week, and 2-year studies. Quantitative neurobehavioral testing was performed during the 14-day, 13-week and 2-year studies. Genetic toxicology studies were conducted for gene mutation induction in *Salmonella typhimurium* and for induction of sister chromatid exchanges in mouse bone marrow cells and of micronuclei from peripheral blood erythrocytes.

14-Day Studies: Groups of five B6C3F$_1$ mice of each sex were exposed to 0, 12, 25, 50, 100, or 200 ppm methyl bromide by inhalation 6 hours per day, 5 days per week for 2 weeks. Only four female mice and one male mouse survived 10 exposures at 200 ppm. No deaths occurred at the lower doses. Neurobehavioral effects including trembling and paralysis were noted in all groups, but were most pronounced in the three highest dose groups. Red urine was noted in the mice exposed to 200 ppm.

13-Week Studies: Groups of 10 mice of each sex were exposed to 0, 10, 20, 40, 80, or 120 ppm methyl bromide by inhalation 6 hours per day, 5 days per week for 13 weeks. Additional groups of eight to 17 mice were concurrently exposed for neurobehavioral and genetic toxicology studies. The final mean body weight of males exposed to 120 ppm was significantly (12%) lower than that of the controls. Four of 24 males exposed to 120 ppm died during the study.

Groups of 10 rats of each sex were exposed to 0, 30, 60, or 120 ppm methyl bromide by inhalation 6 hours per day, 5 days per week for 13 weeks. Additional groups of eight rats were concurrently exposed for neurobehavioral studies. Final mean body weights of rats exposed to 120 ppm were 12% lower than those of the controls for males and 13% lower for females. No rats died as a result of methyl bromide exposure during the studies.

Special 6-Week Target Organ Toxicity Studies: Neither the 14-day nor the 13-week studies provided strong evidence for specific organ toxicity. Six-week studies were therefore conducted to identify target organs for the 2-year studies. Groups of 20 rats and mice of each sex were exposed to methyl bromide by inhalation for 6 hours per day, 5 days per week for 6 weeks at a dose of 160 ppm. Mortality rates exceeded 50% in the male mice after eight exposures, in female mice after six exposures, and in male rats after 14 exposures. Only the female rat group survived 30 exposures with less than 50% mortality. The study identified the brain, kidney, nasal cavity, heart, adrenal gland, liver, and testis as the primary organs to examine for toxicity in the 2-year methyl bromide inhalation studies.

2-Year Studies: Groups of 70 B6C3F$_1$ mice of each sex were exposed to methyl bromide by inhalation at 0, 10, 33, or 100 ppm for 6 hours per day, 5 days per week for up to 103 weeks. Additional groups of 16 mice were included for neurobehavioral evaluations throughout the 2-year studies. By 20 weeks (139 days), 27 males and 7 females exposed to 100 ppm had died and methyl bromide exposure was discontinued for the remaining mice in this dose group. Ten female mice from the 100 ppm group predesignated for the 15-month interim evaluation were killed on schedule and all other high-dose animals were allowed to live to term (24 months) for evaluation of chronic toxicity and carcinogenicity. Clinical signs indicative of neurotoxicity, including tremors, abnormal posture, tachypnea, and hind leg paralysis, persisted in these high-dose mice until the end of the studies.

Final mean body weights of surviving 100 ppm males and females were markedly lower (33% and 31%) than those of the controls. Neurobehavioral changes occurred in male and female mice initially exposed to 100 ppm methyl bromide, with more pronounced changes observed in males. In general, these animals were less active and manifested a heightened sensitivity in the startle response than mice in other dose groups.

Exposure to methyl bromide was not carcinogenic under the conditions of these studies. However, there was an increase in the incidence of several nonneoplastic lesions in the brain, heart, bone (sternum), and nose. Degenerative changes in the cerebellum and cerebrum occurred in males and females exposed to 100 ppm. Myocardial degeneration and cardiomyopathy were observed in the hearts of mice exposed to 100 ppm. An increased incidence of sternal dysplasia was seen in treated animals, particularly in those exposed to 100 ppm. An increased incidence of olfactory epithelial necrosis and metaplasia within the nasal cavity was seen in the mice exposed to 100 ppm, particularly males.

Genetic Toxicology: Methyl bromide was positive for induction of gene mutations in *Salmonella typhimurium* strain TA100, with and without exogenous metabolic activation; negative results were obtained with TA98 in this assay. *In vivo*, methyl bromide induced sister chromatid exchanges in bone marrow cells and micronuclei in peripheral erythrocytes of female mice exposed by inhalation for 14 days. No significant increase in either sister chromatid exchanges or micronuclei was observed in male or female mice exposed to methyl bromide by inhalation for 4, 8, or 12 weeks.

Conclusions: Under the conditions of these 2-year inhalation studies, methyl bromide caused degenerative changes in the cerebellum and cerebrum, myocardial degeneration and cardiomyopathy, sternal dysplasia, and olfactory epithelial necrosis and metaplasia. Toxic effects persisted although exposure to methyl bromide in the 100 ppm group terminated after 20 weeks. There was *no evidence of carcinogenic activity* of methyl bromide in male or female $B6C3F_1$ mice exposed to 10, 33, or 100 ppm.

Synonym: Bromomethane

Report Date: March 1992

TR-386 Toxicology and Carcinogenesis Studies of Tetranitromethane (CAS No. 509-14-8) in F344/N Rats and $B6C3F_1$ Mice (Inhalation Studies)

Tetranitromethane is a volatile contaminant formed during the manufacture of TNT and has been used as a rocket fuel and biochemical reagent. Toxicology and carcinogenesis studies were conducted in F344/N rats and $B6C3F_1$ mice of each sex by whole body exposure to tetranitromethane vapor (greater than 99% pure), 6 hours per day, 5 days per week for 14 days, 13 weeks, or 2 years. Additional groups of male mice were exposed to tetranitromethane for evaluation at 1 year. Genetic toxicology studies were performed in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

Fourteen-Day Studies: Exposure concentrations ranged from 2 to 25 ppm for rats and from 2 to 50 ppm for mice. All rats exposed to 25 ppm and all mice exposed at the top concentration of 50 ppm died by day 2; reduced survival was seen in mice exposed to 25 ppm and in rats exposed to 10 ppm. Pulmonary edema in rats and inflammation of the lung in mice were seen in those animals in the 25- and 50-ppm exposure groups examined microscopically.

Thirteen-Week Studies: Exposure concentrations ranged from 0.2 to 10 ppm for rats and mice. No exposure-related deaths occurred in rats. The final mean body weight of rats exposed to 10 ppm was 16% lower than that of controls for males and 6% lower for females. Exposure-related histologic effects included squamous metaplasia of the respiratory epithelium of the nasal mucosa and chronic inflammation of the lung.

No deaths of mice could be clearly related to exposure to tetranitromethane. The final mean body weights of mice exposed to 5 or 10 ppm were 5% or 12% lower than that of controls for males and 9% or 12% lower for females. Exposure-related histologic effects in mice included inflammation and squamous metaplasia of the respiratory epithelium of the nasal mucosa and hyperplasia of the bronchiolar epithelium.

Based on the incidences and severity of lesions in the respiratory at the higher concentrations used in the 13-week studies, exposure concentrations chosen for the 2-year studies were 0, 2, and 5 ppm for groups of 50 rats of each sex and 0, 0.5, and 2 ppm for groups of 50 mice of each sex. Additional groups of 6 or 10 male mice were exposed at concentrations of 0, 0.5, or 2 ppm for 1 year.

Body Weights and Survival in the Two-Year Studies: Mean body weights of male and female rats exposed to 5 ppm were approximately 5%-15% lower than those of controls after week 70. Survival of rats at 104 weeks was as follows: male: control, 18/50; 2 ppm, 17/50; 5 ppm, 4/50; female: 25/50; 34/50; 15/50; survival of rats at the top concentration was reduced due to neoplasia.

Mean body weights of exposed mice were variable and ranged as much as 10% below those of controls during the second year of the studies. Survival of exposed male mice at 104 weeks was significantly lower than that of controls due to neoplasia (control, 37/50; 0.5 ppm, 26/50; 2 ppm, 15/50). Survival of female mice was not significantly affected by exposure to tetranitromethane (31/50; 28/50; 24/50).

Neoplastic and Nonneoplastic Effects in the Two-Year Studies: Effects of exposure to tetranitromethane were limited to the respiratory tract. Hyperplasia of the alveolar and bronchiolar epithelium was observed at increased incidences in exposed rats. The incidence of alveolar/bronchiolar adenomas and carcinomas were markedly increased in exposed male and female rats, with carcinomas (many of which metastasized to other sites) occurring in nearly all rats exposed to the top concentration of 5 ppm (adenomas or carcinomas—male: control,

1/50; 2 ppm, 33/50; 5 ppm, 46/50; female: 0/50; 22/50; 50/50). Many of the rats exposed to 5 ppm also had squamous cell carcinomas of the lung (male: 0/50; 1/50; 19/50; female: 0/50; 1/50; 12/50).

Hyperplasia of the respiratory epithelium and chronic inflammation of the nasal mucosa were observed at increased incidences in exposed male and female rats. Squamous metaplasia of the respiratory epithelium was increased in exposed male rats. No neoplasms of the nasal passage were seen.

In exposed mice, hyperplasia of the alveolar and bronchiolar epithelium was observed at increased incidences. Alveolar/bronchiolar neoplasms, primarily carcinomas (many of which metastasized to other sites), were increased in exposed male and female mice (male: control, 12/50; 0.5 ppm, 27/50; 2 ppm, 47/50; female: 4/49; 24/50; 49/50).

Chronic inflammation of the nasal mucosa and hyperplasia and squamous metaplasia of the respiratory epithelium of the nasal cavity occurred at increased incidences in female mice exposed to 2 ppm. No primary neoplasms of the nasal passage were observed in mice.

Oncogene Analysis: DNA from 14/19 rat and 4/4 mouse lung neoplasms caused morphologic transformation after transfection into cultured NIH/3T3 fibroblasts. The transforming gene from both rat and mouse lung neoplasms was determined by Southern blot analysis to be an activated K-*ras* oncogene.

Genetic Toxicology: Tetranitromethane was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1535 with and without exogenous metabolic activation (S9); no mutagenic activity was observed in TA1537 with or without S9. Chromosomal aberrations were observed in CHO cells treated in vitro with tetranitromethane in the presence of S9. Sister chromatid exchanges were induced in CHO cells in the absence of S9.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of tetranitromethane for male and female F344/N rats and male and female B6C3F$_1$ mice, based on increased incidences of alveolar/bronchiolar neoplasms in both species and squamous cell carcinomas of the lung in rats.

Chronic inflammation of the nasal mucosa was related to exposure in rats and female mice, and hyperplasia and squamous metaplasia of the respiratory epithelium were increased in exposed male rate.

Synonym: TNM

Report Date: March 1990

TR-387 Toxicology and Carcinogenesis Studies of *dl*-Amphetamine Sulfate (CAS No. 60-13-9) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

dl-Amphetamine sulfate is used for the treatment of narcolepsy in adults and behavioral syndromes in children. Toxicology and carcinogenesis studies were conducted by administering *dl*-amphetamine sulfate (USP grade) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

14-Day Studies: The chemical was administered at dietary concentrations of 0, 47, 94, 188, 375, or 750 ppm for rats and 0, 125, 250, 500, 1,000, or 2,000 ppm for mice. Decreased body weight gain was seen at the higher concentrations, but no chemical-related deaths or toxic lesions were observed.

13-Week Studies: The chemical was administered at dietary concentrations of 0, 47, 94, 188, 375, or 750 ppm for rats and 0, 125, 250, 500, 1,000, or 2,000 ppm for mice. None of the rats died, but 6/10 male mice and 7/10 female mice that received 2,000 ppm, 3/10 male mice that received 1,000 ppm, and 8/10 male mice that received 500 ppm died before the end of the studies. Decreased body weight gain and hyperactivity were seen in dosed rats and mice. Final body weights of rats receiving 188 ppm or more were 62% to 89% those of controls, and final body weights of mice receiving 250 ppm or more were 70% to 86% those of controls. There were no lesions that were considered to be a primary effect of the chemical.

Based on decreased body weight gain and hyperactivity in the 13-week studies, 2-year studies were conducted by feeding diets containing 0, 20 or 100 ppm *dl*-amphetamine sulfate to groups of 50 rats or 50 mice of each sex.

Body Weights and Survival in the 2-Year Studies: No significant differences in survival were observed between any groups of rats or mice (male rats: control, 30/50; low dose, 31/50; high dose, 33/50; female rats: 33/50; 42/50; 37/50; male mice: 48/50; 48/50; 49/50; female mice: 35/50; 36/50; 44/50).

Final body weights of dosed rats and mice were decreased relative to those of controls. Final body weights were 92% and 86% those of controls for low- and high-dose male rats, 89% and 70% those of controls for low- and high-dose female rats, 85% and 72% those of controls for low- and high-dose male mice, and 81% and 66% those of controls for low- and high-dose female mice. Hyperactivity was observed in all dosed groups.

Feed consumption was similar among control and exposed groups with the exception of high-dose female rats (84% of controls) and high-dose male mice, for which hyperactivity resulted in scattering of feed and overestimation of feed consumption. The average amount of *dl*-amphetamine sulfate consumed per day was estimated to be 1 or 5 mg/kg for low- and high-dose rats, 4 or 30 mg/kg for low- or high-dose male mice, and 3 or 19 mg/kg for low- or high-dose female mice.

Nonneoplastic and Neoplastic Effects in the 2-Year Studies: Myelofibrosis, cataracts, and retinal atrophy in female rats, and ovarian atrophy in female mice occurred in a larger proportion of high-dose animals than in controls.

Dose-related increases in neoplasms did not occur in rats or mice receiving amphetamine. The administration of *dl*-amphetamine sulfate was associated with

decreases in the incidence of total neoplasms and in the incidences of certain site-specific neoplasms, including pheochromocytomas of the adrenal gland in male rats (23/49, 15/44, 7/50), fibroadenomas of the mammary gland in female rats (21/50, 11/50, 2/50), adenomas of the anterior pituitary gland in male and female rats and female mice (male rats: 15/49, 15/48, 9/49; female rats: 31/50, 24/48, 19/50; female mice: 12/49, 6/49, 1/46), endometrial stromal polyps of the uterus of female rats (10/50, 6/50, 3/50), adenomas or carcinomas (combined) of the liver in male and female mice (male: 14/50, 12/50, 2/50; female: 5/50, 1/50, 1/47), adenomas of the harderian gland in male and female mice (male: 4/50, 2/50, 0/50; female: 5/50, 2/50, 0/47), and adenomas or carcinomas (combined) of the lung in male and female mice (male: 8/50, 3/50, 4/50; female: 8/50, 6/50, 1/47).

Genetic Toxicology: *dl*-Amphetamine sulfate was tested for induction of gene mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without exogenous metabolic activation (S9); the only response observed was in strain TA98 in the presence of S9, and it was judged to be equivocal. No induction of sister chromatid exchanges or chromosomal aberrations occurred in Chinese hamster ovary cells treated with amphetamine sulfate in either the presence or the absence of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity* of *dl*-amphetamine sulfate for male or female F344/N rats or male or female B6C3F$_1$ mice fed 20 or 100 ppm. The administration of *dl*-amphetamine sulfate was associated with decreased body weight. There were decreased incidences of total neoplasms in dosed rats and mice, of adrenal pheochromocytomas in male rats, of mammary gland fibroadenomas and uterine polyps in female rats, of pituitary gland adenomas in male and female rats and female mice, and of harderian gland adenomas, liver neoplasms, and lung neoplasms in male and female mice.

Synonyms: (±)-amphetamine sulfate; (±)-2-amino-1-phenylpropane sulfate; amphetamine sulfate; deoxynorephedrine; desoxynorephedrine; (±)-α-methylphenethylamine sulfate; (±)-phenisopropylamine sulfate; β-phenyl isopropylamine

Trade Names: Acedron; Adipan; Adiparthrol; Aketdrin; Aktedrin; Alentol; Amfetamina; Amphaetamin; Amphamed; Amphatamin; Amphate; Amphedrine; Amphetaminum; Amphezamin; Amphoids-S; Anara; Anfetamina; Anorexine; Astedin; Benzafinyl; Benzamphetamine; Benzebar; Benzedrina; Benzedryna; Benzolone; Benzpropamine; Betafen; Betaphen; Bluzedrin; Centramina; Didrex; Dietamine; Durophet; Elastonin; Elastonon; Euphobine; Euphodine; Euphodyn; Fabedrine; Fenamin; Fenara; Fenedrin; Fenopromin; Halloo-Wach; Ibiozedrine; Isamin; Isoamin; Isoamyne; Isomyn; Leodrin; Levonor; Linampheta; Mecodrin; Mimetina; Monetamine; Noclon; Norephedrane; Norphedrane; Novydrine; Oktedrin; Oraldrina; Ortenal; Orthedrin; Percomon; Pharmamedrine; Pharmedrine; Phenamine; Phenedrine; Phenopromin; Phenpromin; Profamina; Profetamine; Propenyl; Propisamine; Psychedrine; Psychedryna; Psychedrinum; Psychoton; Racephen; Rhinalator; Sedolin; Simpamina; Simpamine; Simpatedrin; Stimulan; Sympametin; Sympamine; Sympatedrine; Synsatedrine; Theptine; Vapedrine; Weckamine; Zedrine

Slang for Amphetamines: bennies; benzies; cartwheels; hearts; peaches; roses

TR-388 Toxicology and Carcinogenesis Studies of Ethylene Thiourea (CAS: 96-45-7) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

Ethylene thiourea is a white crystalline solid used extensively in the rubber industry as an accelerator in the vulcanization of elastomers. It is also a trace contaminant and metabolic degradation product of a widely used class of ethylene bisdithiocarbamate fungicides. Ethylene thiourea is known to produce thyroid neoplasms in rats and liver neoplasms in mice following long-term administration; thus, it was chosen by the National Toxicology Program in an investigation of the potential value of perinatal exposures in assessing chemical carcinogenicity.

Chronic toxicity and carcinogenicity studies of ethylene thiourea, 99% pure, were conducted in F344/N rats and B6C3F$_1$ mice of each sex. The studies were designed to determine 1) the effects of ethylene thiourea in rats and mice receiving adult exposure only (a typical carcinogenicity study), 2) the toxic and carcinogenic effects of ethylene thiourea on rats and mice receiving perinatal exposure only (dietary exposure of dams prior to breeding and throughout gestation and lactation), and 3) the effects of combined perinatal and adult exposure to ethylene thiourea.

Studies in F344/N Rats: In a preliminary study to determine the perinatal dietary concentrations for the 2-year studies, female F344/N rats were fed 0, 8, 25, 83, or 250 ppm ethylene thiourea in the feed beginning 2 weeks prior to breeding and continuing throughout gestation and lactation, and the pups were fed at these same concentrations up to 9 weeks postweaning. Based on decreased survival of rat pups between postnatal days 0 to 4 and reduction in body weight gains in male weanling rats receiving 250 ppm, dietary concentrations of 0, 9, 30, and 90 ppm were selected for the perinatal (F$_0$) exposure levels in the 2-year studies. Groups of 10 male and 10 female rats, 8 to 9 weeks of age, were fed diets containing 0, 60, 125, 250, 500, or 750 ppm ethylene thiourea for 13 weeks to determine the adult dietary concentrations. Because of reduced weight gains and decreased feed consumption in rats receiving 500 or 750 ppm ethylene thiourea, dietary concentrations of 0, 25, 83, and 250 ppm were selected for the adult (F$_1$) exposure during the 2-year studies.

In the 2-year studies, perinatal and adult exposures to ethylene thiourea were applied separately and together to groups of male or female rats as shown in the following table.

The principal toxic effects of ethylene thiourea involved the thyroid gland. Serum levels of thyroxine (T_4) and/or triiodothyronine (T_3) were significantly decreased in rats receiving adult concentrations of 83 or 250 ppm, and thyrotropin (thyroid-stimulating hormone, TSH) was significantly increased at these concentrations. In male and female rats receiving adult-only exposure of 83 or 250 ppm, the incidences of follicular cell hyperplasia or follicular cell adenoma of the thyroid gland were significantly increased relative to the controls. The incidences of follicular cell carcinoma were significantly increased in the 250 ppm groups, and carcinomas occurred more frequently in males than in females.

Perinatal-only exposure to 90 ppm had no effect on the incidence of thyroid neoplasms in these studies, although there was a marginal increase in follicular cell hyperplasia relative to the controls. However, for groups of rats receiving combined perinatal and adult exposure (F_0:F_1), males and females receiving concentrations of 90:250 ppm ethylene thiourea had significantly increased incidences of thyroid follicular cell neoplasms relative to those receiving adult-only exposure to 250 ppm. Further, groups of male rats receiving 90:83 ppm showed a significantly increased incidence of follicular cell hyperplasia. Final mean body weights of males and survival of males and females receiving combined perinatal (90 ppm) and adult (250 ppm) exposure were lower than those receiving adult-only exposure of 250 ppm.

Thus, in rats, combined perinatal and adult exposure slightly enhanced the toxicity and proliferative effects on the thyroid gland observed with adult-only exposure to ethylene thiourea.

Neoplasms of the Zymbal's gland were marginally increased in rats receiving 90:250 ppm (males - 0:0, 1/50; 90:250, 5/50; females - 0:0, 1/50; 90:250, 4/50). Mononuclear cell leukemia occurred with a significant trend in groups of male and female rats receiving perinatal exposure of 90 ppm and increasing adult concentrations (90:0, 90:83, and 90:250 ppm), and for female rats without perinatal exposure (0:0, 0:83, and 0:250 ppm). The incidences of mononuclear cell leukemia in males receiving 90:83 ppm and males and females receiving 90:250 ppm were statistically significant relative to the respective 0:0 ppm groups. Low incidences of renal tubule cell adenomas occurred in most dose groups of male rats, but not in the highest dose group or the controls.

Studies in B6C3F_1 Mice: In a preliminary study to determine the perinatal dietary concentrations for the 2-year studies, adult female C57BL/6N mice were fed 0, 33, 100, 330, or 1,000 ppm ethylene thiourea in the feed beginning 2 weeks prior to breeding and continuing throughout gestation and lactation and up to 9 weeks postweaning. Because of reduced survival of mouse pups at postnatal day 28 and lower final mean body weights in weanlings receiving perinatal exposure of 1,000 ppm, dietary concentrations of 0, 33, 110, and 330 ppm were selected for the perinatal exposure levels in the 2-year studies. Groups of 10 male and 10 female mice, 8 to 9 weeks of age, were fed diets containing 0, 125, 250, 500, 1,000, or 2,000 ppm ethylene thiourea for 13 weeks to determine the adult dietary concentrations. Moderately severe diffuse follicular cell hyperplasia in the thyroid gland and centrilobular cytomegaly of the liver occurred in mice receiving 2,000 ppm. Because the severity of the thyroid lesion (and degree of hypothyroidism) at this concentration was considered potentially life threatening in 2-year studies, dietary concentrations of 0, 100, 330, and 1,000 ppm ethylene thiourea were selected for adult exposure during the 2-year studies.

In the 2-year studies, perinatal and adult exposures to ethylene thiourea were applied separately and together to groups of male or female mice as shown in the following table.

The principal toxic effects of ethylene thiourea in mice occurred in the thyroid gland, liver, and pituitary gland. Serum levels of T_3 were significantly decreased in groups of mice receiving adult concentrations of 1,000 ppm; TSH was significantly increased in mice receiving 330 and 1,000 ppm. The incidences of follicular cell hyperplasia and neoplasia increased principally in males receiving 1,000 ppm and in females receiving 330 or 1,000 ppm. Follicular cell carcinomas were significantly increased in mice receiving 1,000 ppm. Incidences of centrilobular hepatocellular cytomegaly (males and females), hepatocellular adenoma (females), hepatocellular carcinoma (males and females), and adenoma or carcinoma combined (males and females) also were significantly increased in mice receiving F_1 concentrations of 330 or 1,000 ppm. In the pituitary gland, incidences of focal hyperplasia (males) or adenoma (males and females) of the pars distalis were significantly increased in groups of mice receiving 1,000 ppm ethylene thiourea.

Perinatal exposure to concentrations of 330 ppm had no effect on the incidences of nonneoplastic lesions or neoplasms in mice. For groups of mice receiving combined perinatal and adult exposure, females receiving F_0:F_1 concentrations of 330:330 ppm had significantly increased incidences of follicular cell adenoma relative to those receiving adult-only exposure to 330 ppm. Similarly, male mice receiving F_0:F_1 concentrations of 330:330 ppm had significantly increased incidences of follicular cell hyperplasia. Thus, in mice, perinatal exposure slightly enhanced the proliferative effects on the thyroid gland of adult exposure. There were no effects of perinatal exposure in mice at sites other than in the thyroid gland.

Conclusions: 2-Year Adult-Only Exposure:Under the conditions of these 2-year adult-only dietary exposures, there was *clear evidence of carcinogenic activity* of ethylene thiourea in male and female F344/N rats, as shown by increased incidences of thyroid follicular cell neoplasms. There was *clear evidence of carcinogenic activity* of ethylene thiourea in male and female B6C3F_1 mice as shown by increased incidences of thyroid follicular cell neoplasms, hepatocellular neoplasms, and adenomas of the pars distalis of the pituitary gland.

Nonneoplastic lesions associated with the administration of ethylene thiourea included follicular cell hyperplasia in rats and mice and follicular cell cytoplasmic vacuolation, centrilobular hepatocellular cytomegaly, and focal hyperplasia of the pars distalis of the pituitary gland in mice. Other effects associated with the administration of ethylene thiourea included decreased serum levels of T_4 and/or T_3 in rats and increased serum levels of TSH in rats and mice.

Perinatal-Only Exposure:Perinatal exposure alone had no effect on the incidences of neoplasms in rats or mice after 2 years. Animals may have been able to tolerate higher perinatal exposure concentrations.

Combined Perinatal and 2-Year Adult Exposures: Combined perinatal and 2-year adult dietary exposure to ethylene thiourea confirmed the findings of the 2-year adult-only exposures for the incidences of neoplasms in the thyroid gland of rats and mice and the liver and pituitary gland of mice. In male and female rats, combined perinatal and adult exposure to 90:250 ppm was associated with marginal increases, relative to the untreated (0:0 ppm) controls, in Zymbal's gland neoplasms and mononuclear cell leukemia, which may have been related to chemical administration. In rats receiving adult exposure to 250 ppm ethylene thiourea, perinatal exposure to 90 ppm was associated with a slightly enhanced incidence of thyroid neoplasms compared to adult-only exposure. However, increasing perinatal exposure from 0 to 90 ppm had no effect on incidences of thyroid neoplasms in rats receiving adult exposure to 83 ppm. Increasing perinatal exposure from 0 to 330 ppm was associated with a marginally increased incidence of thyroid neoplasms in female mice receiving adult exposure to 330 ppm, but there were no enhancing effects of perinatal exposure in mice receiving adult exposure to 1,000 ppm.

Synonyms: 2-Imidazolidinethione; Imidazoline-2-thiol; 2-mercaptoimidazoline; *N,N'*-ethylenethiourea; 1,3-ethylenethiourea; 2-imadazoline-2-thiol

Report Date: March 1992

TR-389 Toxicology and Carcinogeneis Studies of Sodium Azide (CAS: 26628-22-8) in F344 Rats (Gavage Studies)

Sodium azide is a white crystalline solid used in the manufacture of the explosive lead azide. It is the principal chemical used to generate nitrogen gas in automobile safety airbags and airplane escape chutes and is a broad-spectrum biocide used in both research and agriculture.

Toxicology and carcinogenicity studies were conducted by administering sodium azide (greater than 99% pure) in distilled water by gavage to groups of male and female F344/N rats once daily, 5 days per week for 14 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

14-Day Studies: Rats received 0, 5, 10, 20, 40, or 80 mg/kg sodium azide. All male and female rats receiving 40 or 80 mg/kg and two of five female rats receiving 20 mg/kg died during the first week of the studies. Clinical findings of toxicity included lethargy and inactivity. No grossly observable lesions were present in any of the dose groups.

13-Week Studies: Rats received 0, 1.25, 2.5, 5, 10, or 20 mg/kg sodium azide. Seven of 9 males and all 10 females receiving 20 mg/kg died before the end of the studies. Final mean body weights of treated rats were within 10% of those of the controls. Compound-related clinical findings of toxicity in the 20 mg/kg dose groups included lethargy and labored breathing. Histopathologic lesions induced by sodium azide were limited to the brain (necrosis of the cerebrum and thalamus) and lung (congestion, hemorrhage, and edema), and were observed in rats receiving 20 mg/kg that died during the studies.

Body Weights, Feed Consumption, and Survival in the 2-Year Studies: Because compound-related deaths were observed in the groups receiving 20 mg/kg in the 13-week studies, lower dose levels were used in the 2-year studies. Two-year studies were conducted by administering 0, 5, or 10 mg/kg sodium azide to groups of 60 male and 60 female rats. Dose-related depression in mean body weight was observed throughout the study period. Mean feed consumption values in low- and high-dose groups were lower than control values. Survival of high-dose rats of each sex was significantly ($P<0.05$) lower than controls (males-control, 24/60; low-dose, 27/60; high-dose, 9/60; females-37/60; 43/60; 21/59). The reduced survival was attributed to brain necrosis and cardiovascular collapse induced by sodium azide.

Neoplastic and Nonneoplastic Effects in the 2-Year Studies: There were no compound-related increases in incidences of neoplasms in rats. Significantly decreased incidences were observed for certain neoplasms, including mononuclear cell leukemia in male rats (control, 33/60; low-dose, 28/60; high-dose, 14/60), adrenal gland pheochromocytoma in male rats (26/55; 16/56; 6/54), mammary gland fibroadenoma in female rats (20/60; 11/60; 8/59), and pituitary gland neoplasms in female rats (37/60; 28/60; 17/59). These decreases reflected to some extent, but could not be attributed solely to, the reduced survival of the high-dose groups. Compound-related nonneoplastic brain lesions (necrosis of the cerebrum and thalamus) were observed at significantly ($P<0.001$) increased incidences in high-dose male and female rats. The increased incidence of lung congestion observed in this dose group was considered due to cardiovascular collapse secondary to brain necrosis.

Genetic Toxicology: Sodium azide was mutagenic in *Salmonella typhimurium* strains TA100 and TA1535, with or without exogenous metabolic activation (S9); it was not mutagenic in strain TA1537 or TA98. In cytogenetic tests with Chinese hamster ovary cells, sodium azide induced sister chromatid exchanges, but not chromosomal aberrations, in the presence and the absence of S9.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of sodium azide in male or female F344/N rats administered 5 or 10 mg/kg.

Sodium azide induced necrosis in the cerebrum and the thalamus of the brain in both male and female rats.

Synonyms: Azide, Azium, Smite

Report Date: September 1991

TR-390 Toxicology and Carcinogenesis Studies of 3,3'-Dimethylbenzidine Dihydrochloride (CAS No. 612-82-8) in F344/N Rats (Drinking Water Studies)

3,3'-Dimethylbenzidine dihydrochloride is one of five chemicals being evaluated in 2-year carcinogenicity and toxicity studies as part of the NTP's Benzidine Dye Initiative. This Initiative was designed to evaluate representative benzidine congeners, benzidine congener-derived dyes, and benzidine-derived dyes. 3,3'-Dimethylbenzidine dihydrochloride was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering 3,3'-dimethylbenzidine dihydrochloride (approximately 99% pure) in drinking water to groups of F344/N rats of each sex for 14 days, 13 weeks, or 9 or 14 months. The 14-month exposures were planned as 24-month exposures but were terminated early because of rapidly declining animal survival, due primarily to neoplasia. These studies were performed only in rats because similar studies were being performed in mice at the National Center for Toxicological Research (NCTR). Hematologic and serum chemical analyses and thyroid hormone determinations were conducted in conjunction with the 13-week and 9-month studies. Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells, and *Drosophila melanogaster*.

14-Day Studies: Rats were exposed to 3,3'-dimethylbenzidine dihydrochloride in drinking water at doses ranging from 600 to 7,500 ppm. All five males and one female in the 7,500 ppm group and 1/5 males in the 5,000 ppm group died. Final mean body weights were decreased in males receiving 1,250 ppm or more and in all exposed females, and final mean body weights of animals receiving 2,500 ppm or more were lower than initial weights. Water consumption decreased with increasing chemical concentration. Compound-related effects observed in rats receiving 5,000 ppm or more included minimal to slight hepatocellular necrosis, accumulation of brown pigment (presumably bile) in individual hepatocytes, increased severity of nephropathy relative to controls, and severe lymphocytic atrophy of the thymus. Treated animals also showed an increased severity of atrophy of the bone marrow relative to controls, varying degrees of lymphocytic atrophy of the mandibular and mesenteric lymph nodes and spleen, increased vacuolization and necrosis of cells of the adrenal cortex, focal acinar cell degeneration in the pancreas, and, in males, increased immature sperm forms in the testis and epididymis.

13-Week Studies: 3,3'-Dimethylbenzidine dihydrochloride was administered in drinking water at doses of 300, 500, 1,000, 2,000, and 4,000 ppm. All rats receiving 4,000 ppm and 4/10 males and 1/10 females receiving 2,000 ppm died before the end of the studies. Depressions in final mean body weight relative to controls ranged from 12% to 48% for males and from 9% to 42% for females. Water consumption decreased with increasing dose. At compound concentrations of 300 to 2,000 ppm, mean water consumption was 29% to 83% of control values. Compound-related effects included an increase in the severity of nephropathy relative to controls; hepatocellular necrosis and accumulation of brown pigment (presumably bile) in sinusoidal lining cells; lymphocytic atrophy of the thymus, spleen, and mandibular and mesenteric lymph nodes; atrophy of the bone marrow in the higher-dose groups; degeneration of pancreatic acinar cells; and, in males, immature sperm forms in the testis and epididymis.

Decreases in serum triiodothyronine (T_3) values were observed in exposed females, and decreases in mean thyroxin (T_4) concentrations in exposed males and females; no significant changes were observed in thyroid stimulating hormone (TSH) levels in exposed rats.

Based on the decreased survival, reductions in water consumption and body weight gain, and chemical-induced hepatocellular and renal lesions observed in the 13-week studies, the doses selected for the 9- and 14-month drinking water studies of 3,3'-dimethylbenzidine dihydrochloride were 0, 30, 70, and 150 ppm. Seventy rats of each sex were used in the control group, 45 in the low-dose group, 75 in the mid-dose group, and 70 in the high-dose group.

9-Month Studies: Ten rats of each sex in the control and 150 ppm dose groups were evaluated after 9 months. Chemical-related effects observed in exposed animals included alveolar/bronchiolar carcinoma in one male, basal cell carcinoma of the skin in one male, a squamous cell carcinoma of the oral cavity in one female, preputial gland carcinoma in two males, clitoral gland carcinoma in three females, adenocarcinoma of the small intestine in two males, Zymbal's gland carcinoma in two males and three females, hepatocellular carcinoma in two males, and adenomatous polyps of the large intestine in three males. Other effects seen in dosed rats included focal cellular alteration in the liver, lymphoid atrophy in the spleen, and increased severity of nephropathy relative to controls. An increase in serum T3 values was observed in exposed males, and a decrease in mean T4 concentrations in exposed males and females. TSH concentrations were increased in exposed male and female rats.

Body Weights and Survival in the 14-Month Studies: The average amount of 3,3'-dimethylbenzidine

dihydrochloride consumed per day was approximately 1.8, 4.0, or 11.2, mg/kg for low-, mid-, or high-dose male rats and 3.0, 6.9, or 12.9 mg/kg for low-, mid-, or high-dose female rats. The mean body weight of high-dose males was about 85% of the control value by week 28. By the end of the study, mean body weights of low-, mid-, and high-dose males were 97%, 92%, and 70% of the control values, respectively. Mean body weights of high- and mid-dose females were about 85% of the control values at week 32 and week 44, respectively. At the end of the study, mean body weights of exposed females were about 94%, 81%, and 74% of the control values for low-, mid-, and high-dose groups, respectively. Because of extensive neoplasia, many exposed males and females were dying or were sacrificed moribund in the first year, and all high-dose males died by week 55. The studies were terminated at weeks 60 to 61, at which time the group survivals were male: control, 60/60, low dose, 41/45; mid dose, 50/75; high dose, 0/60; female: 59/60; 39/45; 32/75; 10/60.

Nonneoplastic Effects in the 14-Month Studies: Increases in nonneoplastic lesions in dosed rats included cystic degeneration and foci of cellular alteration in the liver; exacerbation of nephropathy; and focal or multifocal hyperplasia of the Zymbal's gland, preputial and clitoral glands, and alveolar epithelium.

Neoplastic Effects in the 14-Month Studies: Neoplasms were observed in exposed rats at many sites: skin, Zymbal's gland, preputial and clitoral glands, liver, oral cavity, small and large intestine, mammary gland, lung, brain, and mesothelium. The incidence of these neoplastic effects in male and female rats is summarized in the table at the end of this section (see page 8 of the Technical Report).

Genetic Toxicology: 3,3'-Dimethylbenzidine dihydrochloride was mutagenic in *Salmonella typhimurium* strain TA98 with exogenous metabolic activation; it was not mutagenic in strains TA100, TA1535, or TA97 with or without activation. 3,3'-Dimethylbenzidine dihydrochloride induced sister-chromatid exchanges (CHO) and chromosomal aberrations in CHO cells in the absence of exogenous metabolic activation; these effects were not evident in test with S9 activation. Sex-linked recessive lethal mutations were induced in germ cells of adult male *Drosophila melanogaster* administered 3,3'-dimethylbenzidine dihydrochloride in feed or by injection. No reciprocal translocations occurred in D. melanogaster germ cells following exposure to 3,3'-dimethylbenzidine dihydrochloride.

Conclusions: Under the conditions of these 14-month drinking water studies, there was *clear evidence of carcinogenic activity* of 3,3'-dimethylbenzidine dihydrochloride for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, small and large intestine, lung, and mesothelium. Increased incidences of neoplasms of the brain may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, mammary gland, and lung. Increased incidences of neoplasms of the brain and mononuclear cell leukemia may have been related to chemical administration.

Synonyms: o-tolidine dihydrochloride; 3,3'-dimethylbiphenyl-4,4'-diamine dihydrochloride; 3,3'-dimethylbiphenyl-4,4'-biphenyldiamine dihydrochloride; 4,4'-diamino-3,3'-dimethylbiphenyl dihydrochloride

Report Date: June 1991

TR-391 Toxicology and Carcinogenesis Studies of Tris(2-chloroethyl) Phosphate (CAS No. 115-96-8) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Tris(2-chloroethyl) phosphate (TRCP), a flame-retardant plasticizer used in plastics, polymeric foams, and synthetic fibers, was studied as part of the National Toxicology Program's class study of trisalkyl phosphate flame retardants. Toxicology and carcinogenesis studies were conducted by administering TRCP (approximately 98% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex for 16 days, 16 weeks, or 2 years. Genetic toxicology studies were performed in *Salmonella typhimurium* and Chinese hamster ovary (CHO) cells.

16-Day Studies: There were no chemical-related deaths, differences in final mean body weight, or histopathological lesions in rats receiving 22 to 350 mg/kg TRCP or in mice receiving 44 to 700 mg/kg TRCP for 12 doses over 16 days. Serum cholinesterase activity in female rats receiving 175 or 350 mg/kg TRCP was reduced slightly (80% of control levels), but enzyme activity in dosed male rats and in mice was similar to that in controls.

16-Week Studies: Rats received 22 to 350 mg/kg TRCP for 16 weeks (female) or 18 weeks (male). Several male and female rats in the 175 or 350 mg/kg dose groups died from chemical toxicity. Final mean body weights of female rats receiving 350 mg/kg were 20% greater than those of controls; final mean body weights of the remaining groups of dosed female rats and dosed male rats were similar. Chemical-related neuronal necrosis occurred in the hippocampus and thalamus of female rats and, to a lesser extent, of male rats. Serum cholinesterase activity was reduced in females receiving 175 or 350 mg/kg TRCP.

There were no chemical-related deaths, differences in final mean body weight, or differences in cholinesterase activity in mice receiving 44 to 700 mg/kg TRCP for 16 weeks. Tubule epithelial cells with enlarged nuclei (cytomegaly and karyomegaly) were observed in the kidneys of high-dose (700 mg/kg) male and female mice.

2-Year Studies: The 2-year studies in rats were conducted by administering 0, 44, or 88 mg/kg TRCP to groups of 60 males and females, 5 days per week for up to 104 weeks; 9 or 10 rats of each dose group were evaluated at 66 weeks. The survival of high-dose male and female

rats was reduced relative to that of controls. Final mean body weights of surviving rats were similar to those of controls. The principal chemical-related effects occurred in the kidney and brain of dosed rats. Focal hyperplasia of the renal tubule epithelium and renal tubule adenomas were markedly increased in male rats receiving 88 mg/kg TRCP and, to a lesser extent, in female rats (renal tubule hyperplasia, male rats: 0/50; 2/50; 24/50; female rats: 0/50; 3/50; 16/50; renal tubule adenoma, male rats: 1/50; 5/50; 24/50; female rats: 0/50; 2/50; 5/50). Renal tubule carcinomas occurred in one control and one high-dose male rat. Degenerative lesions consisting of gliosis, mineralization, hemorrhage, and/or hemosiderin accumulation occurred in the cerebrum and brain stem of more than 50% of female rats receiving 44 or 88 mg/kg TRCP; similar lesions were seen in only a few dosed males. Slightly increased incidences of thyroid gland follicular cell neoplasms (male rats: 5/50; 14/50; 13/50; female rats: 14/50; 16/50; 20/50) occurred in dosed males and females, but it is uncertain whether these were related to chemical administration.

The 2-year studies in mice were conducted by administering 0, 175, or 350 mg/kg TRCP to groups of 60 males and females, 5 days per week for up to 104 weeks; 8 to 10 mice of each sex per dose group were evaluated at 66 weeks. There were no significant differences in survival between dosed and control groups of either sex, and final mean body weights of mice were similar among all groups. The principal chemical-related effects occurred in the kidney, in which nuclear enlargement (karyomegaly) of tubule epithelial cells was present in approximately 80% of high-dose mice. In the original diagnosis, renal tubule adenomas were seen in one control male, one high-dose male, and one low-dose female. A carcinoma was also seen in one high-dose male. In a subsequent examination of step sections of all the mouse kidneys, adenomas were found in one low-dose male and two high-dose males. The incidences of renal tubule neoplasms in the original and step sections combined were 1/50, 1/50, and 4/50 for males. Female mice receiving TRCP demonstrated a marginally increased incidence of neoplasms (primarily adenomas) of the harderian gland (3/50; 8/50; 7/50); in addition, three harderian gland neoplasms occurred in high-dose female mice evaluated after 66 weeks.

Genetic Toxicology: TRCP was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 with or without exogenous metabolic activation (S9), and it tested negative for the induction of chromosomal aberrations in Chinese hamster ovary (CHO) cells. TRCP produced an equivocal response in the presence of S9 for the induction of sister chromatid exchanges (SCE) in CHO cells.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity* for male and female F344/N rats receiving tris(2-chloroethyl) phosphate as shown by increased incidences of renal tubule adenomas. Thyroid follicular cell neoplasms and mononuclear cell leukemia in male and female rats may have been related to chemical administration. There was *equivocal evidence of carcinogenic activity* for male $B6C3F_1$ mice as shown by a marginally increased incidence of renal tubule cell neoplasms. There was *equivocal evidence of carcinogenic activity* for female $B6C3F_1$ mice as shown by a marginally increased incidence of harderian gland adenomas.

Renal tubule cell hyperplasia in male and female rats and gliosis, hemorrhage, pigmentation (hemosiderin accumulation), and mineralization in the brains of female rats were associated with the administration of tris(2-chloroethyl) phosphate. Karyomegaly of renal tubule epithelial cells in male and female mice was also chemical related.

Synonyms: 2-chloroethanol phosphate (3:1); tris(β-chloroethyl) phosphate

Trade Names: Fyrol CEF; Disflamoll TCA; NIAX flame retardant

Report Date: May 1991

TR-392 Toxicology and Carcinogenesis Studies of Chlorinated Water (CAS Nos. 7782-50-5 and 7681-52-9) and Chloraminated Water (CAS No. 10599-90-3) (Deionized and Charcoal-Filtered) in F344/N Rats and $B6C3F_1$ Mice (Drinking Water Studies)

Chlorine and chloramine are used as disinfectants in water supplies to prevent the spread of waterborne diseases. The U.S. Environmental Protection Agency and the U.S. Congress, through the Safe Drinking Water Acts and Amendments, initiated studies to determine the most effective way to disinfect water supplies and, at the same time, minimize any potential long-term health effects associated with direct chemical exposure or indirect chemical exposure through the formation of byproducts. As part of this evaluation, 2-year studies of chlorinated or chloraminated deionized charcoal-filtered drinking water were conducted in F344/N rats and $B6C3F_1$ mice to determine the potential toxicity and carcinogenicity associated with prolonged exposure and eliminate possible confounding effects of byproducts of chlorination.

Chlorinated Water Studies: Water containing 0, 70, 140, or 275 ppm chlorine (based on available atomic chlorine) was provided to groups of 70 F344/N rats or $B6C3F_1$ mice of each sex for up to 2 years. Groups of 10 rats or mice of each sex were predesignated for evaluation at 14 or 15 weeks and 66 weeks. Survival at 2 years of rats and mice receiving chlorinated water was similar to that of the controls. Mean body weights of dosed male rats, high-dose female rats, and dosed mice were slightly lower than those of their respective control groups. There was a dose-related decrease in water consumption by rats and mice. Water consumption by high-dose rats during the second year of the studies was 21% lower than

controls for males and 23% lower than controls for females; water consumption by high-dose mice was 31% lower than controls for males and 26% lower than controls for females.

The incidence of mononuclear cell leukemia in mid-dose, but not high-dose, female rats was significantly higher than that in controls (control, 8/50; low-dose, 7/50; mid-dose, 19/51; high-dose, 16/50). The proportion of female rats that died of leukemia before the end of the study and the mean time for observation of animals dying with leukemia were similar among all dose groups and controls. Although the marginal increase in leukemia incidence in the mid- and high-dose female rats suggested a possible association with the administration of chlorinated water, the incidence of leukemia was not clearly dose related. There was no indication of reduced latency of leukemia, and the incidence of leukemia in concurrent controls was less than the mean for historical controls; furthermore, there was no supporting evidence of an effect in male rats. Thus, the marginal increase in leukemia incidence in female rats was considered equivocal evidence of carcinogenic activity. There were no neoplasms or nonneoplastic lesions in male rats or in male or female mice that were clearly associated with the consumption of chlorinated water.

Chloraminated Water Studies: Water containing 50, 100, or 200 ppm chloramine was provided to groups of 70 F344/N rats or B6C3F$_1$ mice of each sex for up to 2 years. The same control groups were used for the chlorinated water and chloraminated water studies. Groups of 9 or 10 rats or mice of each sex were evaluated at 14 or 15 weeks and 66 weeks.

Survival at 2 years of rats and mice receiving chloraminated water was similar to that of the controls. Mean body weights of high-dose rats and dosed mice were lower than those of their respective control groups. There was a dose-related decrease in water consumption by rats and mice. Water consumption during the second year of the studies by high-dose rats was 34% lower than controls for males and 31% lower than controls for females; water consumption by high-dose mice was 42% lower than controls for males and 40% lower than controls for females.

Mononuclear cell leukemia occurred with a marginally increased incidence in the mid- and high-dose female rats receiving chloraminated water (control, 8/50; low dose, 11/50; mid dose, 15/50; and high dose, 16/50). As in female rats receiving chlorinated water, the proportion of female rats that died of leukemia before the end of the study and the mean time for observation of animals dying with leukemia were similar among all dose groups and controls. The marginal increase in leukemia incidence in females receiving chloraminated water was considered equivocal evidence of carcinogenic activity for the same reasons given for female rats receiving chlorinated water. There were no neoplasms or nonneoplastic lesions in male rats or in male or female mice that were clearly associated with the consumption of chloraminated water.

Conclusions: Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of chlorinated water in male F344/N rats receiving 70, 140, or 275 ppm. There was *equivocal evidence of carcinogenic activity* of chlorinated water in female F344/N rats based on an increase in the incidence of mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of chlorinated water in male or female B6C3F$_1$ mice receiving 70, 140, or 275 ppm.

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* of chloraminated water in male F344/N rats receiving 50, 100, or 200 ppm. There was *equivocal evidence of carcinogenic activity* of chloraminated water in female F344/N rats based on an increase in the incidence of mononuclear cell leukemia. There was *no evidence of carcinogenic activity* of chloraminated water in male or female B6C3F$_1$ mice receiving 50, 100, or 200 ppm.

Report Date: March 1992

TR-393 Toxicology and Carcinogenesis Studies of Sodium Fluoride (CAS No. 7681-49-4) in F344/N Rats and B6C3F$_1$ Mice (Drinking Water Studies)

Sodium fluoride is a white, crystalline, water-soluble powder used in municipal water fluoridation systems, in various dental products, and in a variety of industrial applications. Toxicology and carcinogenesis studies were conducted with F344/N rats and B6C3F$_1$ mice of each sex by incorporating sodium fluoride into the drinking water in studies lasting 14 days, 6 months, and 2 years. In addition, genetic toxicology studies were performed with *Salmonella typhimurium*, with mouse L5178Y cells, and with Chinese hamster ovary cells.

14-Day Studies: Rats and mice received sodium fluoride in drinking water at concentrations as high as 800 ppm. (Concentrations are expressed as sodium fluoride; fluoride ion is 45% of the sodium salt by weight.) In the high-dose groups, 5/5 male and 5/5 female rats and 2/5 male mice died; one female rat was given 400 ppm in the drinking water also died before the end of the studies. No gross lesions were attributed to sodium fluoride administration.

6-Month Studies: Rats received concentrations of sodium fluoride in drinking water as high as 300 ppm, and mice as high as 600 ppm. No rats died during the studies; however, among the mice, 4/9 high-dose males, 9/11 high-dose females, and 1/8 males in the 300 ppm group died before the end of the studies. Weight gains were less than those of controls for rats receiving 300 ppm and mice receiving 200 to 600 ppm.

The teeth of rats and mice receiving the higher doses of sodium fluoride were chalky white and chipped or showed unusual wear patterns. Mice and male rats given the higher concentrations had microscopic focal degeneration of the enamel organ. Rats receiving 100 or 300 ppm sodium fluoride had minimal hyperplasia of the gastric

mucosa of the stomach, and one high-dose rat of each sex had an ulcer. Acute nephrosis and/or lesions in the liver and myocardium were observed in mice that died early, and minimal alterations in bone growth/remodeling were observed in the long bones of mice receiving sodium fluoride at concentrations of 50 to 600 ppm.

The sodium fluoride concentrations selected for the 2-year studies in both rats and mice were 0, 25, 100, and 175 ppm in the drinking water. These concentrations were selected based on the decreased weight gain of rats at 300 ppm and of mice at 200 ppm and above, on the incidence of gastric lesions in rats at 300 ppm in the 6-month studies, and on the absence of significant toxic effects at sodium fluoride concentrations as high as 100 ppm in an earlier 2-year study.

Body Weights and Survival in the 2-Year Studies: Mean body weights of dosed and control groups of rats and mice were similar throughout the 2-year studies. Survival of rats and mice was not affected by sodium fluoride administration. Survival rates after 2 years were: male rats-control, 42/80; 25 ppm, 25/51; 100 ppm, 23/50; 175 ppm, 42/80; female rats-59/80; 31/50; 34/50; 54/81; male mice-58/79; 39/50; 37/51; 65/80; female mice-53/80; 38/52; 34/50; 52/80.

Neoplastic and Nonneoplastic Effects in the 2-Year Studies: The teeth of rats and mice has a dose-dependent whitish discoloration, and male rats had an increased incidence of tooth deformities and attrition leading on occasion to malocclusion. The teeth of male and, to a lesser degree, female rats had areas of microscopic dentine dysplasia and degeneration of ameloblasts. Dentine dysplasia occurred in both dosed and control groups of male and female mice; the incidence of this lesion was significantly greater in high-dose than in control male mice. Osteosclerosis of long bones was increased in female rats given drinking water containing 175 ppm sodium fluoride. No other significant nonneoplastic lesions in rats or mice appeared related to sodium fluoride administration.

Osteosarcomas of bone were observed in 1/50 male rats in the 100 ppm group and in 3/80 male rats in the 175 ppm group. None were seen in the control or 25 ppm dose groups. One other 175 ppm male rat had an extraskeletal osteosarcoma arising in the subcutaneous tissue. Osteosarcomas occur in historical control male rats at an incidence of 0.5% (range 0-6%). The historical incidence is not directly comparable with the incidences observed in this study because examination of bone was more comprehensive in the sodium fluoride studies than in previous NTP studies of other chemicals, and the diet used in previous studies was not controlled for fluoride content. In the current study, although the pairwise comparison of the incidence in the 175 ppm group versus that in the controls was not statistically significant, osteosarcomas occurred with a statistically significant dose-response trend, leading to the conclusion that a weak association may exist between the occurrence of these neoplasms and the administration of sodium fluoride. No other neoplastic lesions in rats or mice were considered possibly related to chemical administration.

Genetic Toxicology: Sodium fluoride was negative for gene mutation induction in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with and without S9. In two laboratories, sodium fluoride was tested for induction of trifluorothymidine resistance in mouse L5178Y lymphoma cells; results were positive both with and without S9. Sodium fluoride was tested for cytogenetic effects in Chinese hamster ovary (CHO) cells in two laboratories. In the first laboratory, the sister chromatid exchange (SCE) test was negative with and without S9, and the chromosomal aberration (Abs) test was positive in the absence of S9; in the second laboratory, the SCE test was positive with and without S9, but no induction of Abs was observed. The laboratory that reported a negative result for Abs tested at doses below that shown to be positive at the other laboratory. Similarly, the positive SCE result was obtained at a higher dose and longer harvest time than used by the laboratory reporting the negative SCE response.

Conclusions: Under the conditions of these 2-year dosed water studies, there was *equivocal evidence of carcinogenic activity* of sodium fluoride in male F344/N rats, based on the occurrence of a small number of osteosarcomas in dosed animals. "Equivocal evidence" is a category for uncertain findings defined as studies that are interpreted as showing a marginal increase of neoplasms that may be related to chemical administration. There was *no evidence of carcinogenic activity* in female F344/N rats receiving sodium fluoride at concentrations of 25, 100, or 175 ppm (11, 45, or 79 ppm fluoride) in drinking water for 2 years. There was *no evidence of carcinogenic activity* of sodium fluoride in male or female mice receiving sodium fluoride at concentrations of 25, 100, or 175 ppm in drinking water for 2 years.

Dosed rats had lesions typical of fluorosis of the teeth and female rats receiving drinking water containing 175 ppm sodium fluoride had increased osteosclerosis of long bones.

Report Date: December 1990

TR-394 Toxicology and Carcinogenesis Studies of Acetaminophen (CAS No. 103-90-2) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-395 Toxicology and Carcinogenesis Studies of Probenecid (CAS No. 57-66-9) in F344/N Rats and B6C3F$_1$ (Gavage Studies)

Probenecid is a white crystalline solid commonly used as a uricosuric agent in the treatment of gout. Because of its inhibitory effects on renal tubule transport processes,

probenecid is also used as a therapeutic adjunct to enhance blood levels of penicillin and its action. Toxicology and carcinogenicity studies were conducted by administering probenecid (>99% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex once daily, 5 days per week in 14-day, 13-week, and 2-year studies. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

14-Day Studies: Doses used in the 14-day studies for both rats and mice were 0, 200, 400, 800, 1,600, or 3,200 mg/kg. Of the animals receiving 3,200 mg/kg, all rats, all female mice, and two of five male mice died during the studies. No deaths occurred among the other dose groups. There was a significant reduction in body weight gain in male and female rats receiving 1,600 mg/kg and in female rats receiving 800 mg/kg. No gross lesions were attributed to probenecid administration in rats or mice of either sex.

13-Week Studies: Doses used in the 13-week studies were 0, 50, 100, 200, 400, or 800 mg/kg for rats and 0, 100, 200, 400, 800, or 1,600 mg/kg for mice. No rats died during the 13-week studies. In mice, 5 of 10 males and 3 of 10 females receiving 1,600 mg/kg and 1 of 10 males receiving 800 mg/kg died during the study. Significant reductions in body weight gain occurred in male rats administered 800 mg/kg, male mice administered 1,600 mg/kg, and female mice administered 800 or 1,600 mg/kg. All dose groups of male rats and all groups of female rats receiving 100 mg/kg or more showed significant increases in absolute and/or relative liver weights compared to control groups. This change was also seen in mice receiving 200 mg/kg and greater, except female mice in the 400 mg/kg group. No compound-related lesions occurred in rats or mice of either sex.

Based on compound-related deaths and suppression of body weight gains observed at higher doses in the 13-week studies, doses of 0, 100, and 400 mg/kg were used for the 2-year studies in rats and mice. These doses were administered once daily, 5 days a week for up to 103 weeks to groups of 50 males or 50 females of each species.

Body Weight and Survival in the 2-Year Studies: The mean body weight of high-dose female rats was 10% to 20% lower than that of controls throughout the studies. Mean body weights for all other dosed rats and for all dosed mice were similar to those of controls throughout the 2-year studies.

Survival of high-dose male rats and high-dose and low-dose male mice was significantly lower than that of controls. Survival rates after 2 years were: male rats—control, 37/50; 100 mg/kg, 34/50; 400 mg/kg, 22/50; female rats—24/50; 35/50; 19/50; male mice—38/50; 23/50; 24/50; female mice—32/49; 32/49; 32/50.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: No chemical-related histopathologic toxic effects or increased incidence of tumors attributable to probenecid were observed in male or female rats receiving probenecid by corn oil gavage for up to 2 years. Mammary gland fibroadenomas and combined thyroid C-cell adenomas or carcinomas exhibited significant negative trends in female rats. These decreased tumor rates were associated with lower body weights. The incidence of adrenal medullary pheochromocytomas was significantly decreased in high-dose male rats. No compound-related increase in nonneoplastic lesions was observed in rats of either sex.

No compound-related neoplastic effects were observed in male mice. In high-dose female mice, there were significant increases in the incidences of hepatocellular adenomas (3/48; 2/49; 14/49), but there was no corresponding increase in carcinomas (2/48; 2/49; 3/49). Treatment-related increased incidences of ovarian abscesses in female mice were causally related to *Klebsiella* species infection rather than directly related to chemical administration.

Genetic Toxicology : Probenecid was not mutagenic in *Salmonella typhimurium* strain TA100, TA1535, TA1537, or TA98 with or without metabolic activation. In cytogenetic tests with Chinese hamster ovary cells, probenecid induced sister chromatid exchanges in the absence, but not in the presence of S9 activation. No induction of chromosomal aberrations was observed with or without S9.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of probenecid for male or female F344/N rats receiving 100 or 400 mg/kg in corn oil. There was *no evidence of carcinogenic activity* of probenecid for male B6C3F$_1$ mice given 100 or 400 mg/kg probenecid in corn oil. There was *some evidence of carcinogenic activity* of probenecid for female B6C3F$_1$ mice based on an increased incidence of hepatocellular adenomas.

Synonyms: 4-[(Dipropylamino)sulfonyl]benzoic acid; *p*-(dipropylsulfamoyl)benzoic acid; *p*-(dipropylsulfamyl)benzoic acid

Trade Names: Benacen; Benemid; Benemide; Benn; Probalan; Probecid; Proben; Probenid; Robenecid; Uricocid

Report Date: September 1991

TR-396 Toxicology and Carcinogenesis Studies of Monochloroacetic Acid (CAS No. 79-11-8) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

Monochloroacetic acid, a colorless crystalline material, is used as a postemergence contact herbicide and as an intermediate in the synthesis of other organic compounds. Toxicology and carcinogenicity studies were conducted by administering monochloroacetic acid (99% pure) in deionized water by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex once daily, 5 days per week for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma L5178Y cells, Chinese hamster ovary cells, and *Drosophila melanogaster*.

16-Day Studies: Groups of five rats of each sex received 0, 7.5, 15, 30, 60, or 120 mg monochloroacetic acid/kg body

weight. Doses administered to mice were 0, 15, 30, 60, 120, or 240 mg/kg to groups of five males and 0, 30, 60, 120, 240, or 480 mg/kg to groups of five females. One of five male rats given 120 mg/kg died during the studies. Clear nasal discharge, lacrimation, or both, were observed in all groups of male and female rats receiving monochloroacetic acid. No compound-related gross lesions were observed in rats. All male mice given 240 mg/kg and all females given 240 or 480 mg/kg died during the studies. Hypoactivity, piloerection, ataxia, and lacrimation were observed in mice given 240 or 480 mg/kg. No compound-related gross lesions were observed in mice at necropsy.

13-Week Studies: Groups of 20 rats of each sex received 0, 30, 60, 90, 120, or 150 mg/kg monochloroacetic acid, and groups of 20 mice of each sex received doses of 0, 25, 50, 100, 150, or 200 mg/kg. Three to five animals in each dose group were killed at weeks 4 and 8 for the evaluation of hematology parameters. Compound-related deaths occurred in rats in the three highest dose groups (all males given 120 or 150 mg/kg, 9/10 males given 90 mg/kg, and all females given 90 to 150 mg/kg) and in mice given 200 mg/kg (all males and 2/10 females). Final mean body weights of surviving rats and mice receiving monochloroacetic acid were similar to those of controls. In rats, dose-related increases in blood urea nitrogen, alanine aminotransferase, and aspartate aminotransferase levels were observed, and relative liver and kidney weights were elevated. There were no compound-related changes in the various hematologic or clinical pathology parameters in mice. A dose-related increase in the incidence and severity of cardiomyopathy was observed in male and female rats receiving monochloroacetic acid, and hepatocellular cytoplasmic vacuolization was observed in the high-dose mice that died during the studies.

2-Year Studies: Based on the mortality and compound-related histopathologic lesions observed in the 13-week studies, doses selected for the 2-year studies of monochloroacetic acid were 0, 15, or 30 mg/kg, administered to groups of 70 rats of each sex, and 0, 50, or 100 mg/kg, administered to groups of 60 mice of each sex. Interim evaluations were conducted on 10 rats per dose group after 6 months of treatment with monochloroacetic acid and on seven rats per dose group after 15 months of treatment.

Body Weight and Survival in the 2-Year Studies: Mean body weights of low- and high-dose female and low-dose male rats receiving monochloroacetic acid were within 10% of those of controls throughout the studies; however, after week 30, the mean body weights of high-dose male rats were 4% to 8% less than those of controls. In mice, the mean body weights of dosed males were similar to controls, but those of low- and high-dose females were 6% to 10% less than control values after week 52. Survival of high-dose male and dosed female rats and high-dose male mice was significantly lower than that of controls (male rats: control, 27/53; low-dose, 21/53; high-dose, 16/53; female rats: 37/53; 19/53; 26/53; male mice: 46/60; 39/60; 21/60; female mice: 42/60; 40/60; 44/60).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: There was no compound-related increase in the incidence of neoplasms or nonneoplastic lesions in rats given monochloroacetic acid for 2 years. The incidence of uterine stromal polyps in low- and high-dose female rats was slightly higher than that in controls (2/60; 7/57; 10/60). However, the incidence in the controls was unusually low, and those in the dosed groups were well within the range for NTP historical controls (mean: 21%, range: 10%-38%). Further, because the only malignant stromal neoplasm occurred in a control animal, the polyps were not considered to be related to the administration of monochloroacetic acid. Similarly, there was no monochloroacetic acid-related increase in the incidence of neoplasms in male or female mice, and malignant lymphoma occurred with a significant negative trend in dosed female mice. Increases in the incidence of inflammation of the mucosa of the nasal passages, respiratory epithelial metaplasia of the olfactory epithelium of the nose, and focal squamous cell hyperplasia of the forestomach occurred in dosed male and female mice.

Genetic Toxicology: Monochloroacetic acid was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without exogenous metabolic activation (S9). It induced trifluorothymidine resistance in L5178Y cells in the absence of S9 and induced sister chromatid exchanges without S9 in Chinese hamster ovary cells. Monochloroacetic acid did not induce a significant increase in chromosomal aberrations in Chinese hamster ovary cells, with or without S9. Monochloroacetic acid administered in feed was negative for the induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster;* however, when it was administered by injection, the results were equivocal.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* for monochloroacetic acid in male or female F344/N rats given 15 or 30 mg/kg. There was *no evidence of carcinogenic activity* for monochloroacetic acid in male or female $B6C3F_1$ mice given 50 or 100 mg/kg.

Monochloroacetic acid administration was associated with inflammatory lesions of the nasal mucosa, metaplasia of the olfactory epithelium, and squamous cell hyperplasia of the forestomach in male and female mice.

Synonyms: Chloroacetic acid, α-chloroacetic acid, chloroethanoic acid

Report Date: January 1992

TR-397 Toxicology and Carcinogenesis Studies of C.I. Direct Blue 15 (CAS No. 2429-74-5) in F344 Rats (Drinking Water Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-398 Toxicology and Carcinogenesis Studies of Polybrominated Biphenyl Mixture (Firemaster FF-1) (CAS No. 67774-32-7) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

Note: Polybrominated Biphenyl Mixture (Firemaster FF-1) was previously tested in F344 rats and B6C3F$_1$ mice administered in feed (See TR-244, reported 1983).

TR-399 Toxicology and Carcinogenesis Studies of Titanocene Dichloride (CAS No. 1271-19-8) in F344/N Rats (Gavage Studies)

Titanocene dichloride is an organometallic compound composed of two cyclopentadienyl rings, titanium, and chloride. It is used as a cocatalyst in polymerization reactions. Toxicology and carcinogenesis studies were conducted by administering titanocene dichloride (greater than 98% pure) in corn oil by gavage to groups of F344/N rats for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and in Chinese hamster ovary cells.

14-Day and 13-Week Studies: In the 14-day studies, titanocene dichloride was administered at doses of 0, 65, 125, 250, 500, or 1,000 mg/kg. All high-dose rats and four of the five male and two of the five female rats given 500 mg/kg died during the studies. A dose-related decrease in body weight gain was seen in rats given 125, 250, 500, and 1,000 mg/kg. Lesions related to chemical administration included hepatocellular necrosis, tubule necrosis in the kidney, erosions and ulcers of the glandular stomach, and hyperplasia of the forestomach epithelium.

The 13-week studies were conducted by administering titanocene dichloride at doses of 0, 8, 16, 31, 62, or 125 mg/kg. One female rat in the 125 mg/kg dose group died from chemical toxicity during the fourth week of the studies. Body weight gain was lower in rats given 62 or 125 mg/kg than in control groups. Treatment-associated histopathologic lesions were seen in the stomachs of high-dose males and all groups of females given titanocene dichloride. These lesions included hyperplasia and metaplasia of the glandular stomach and hyperplasia and hyperkeratosis of the forestomach.

Body Weight and Survival in the 2-Year Studies: The doses selected for the 2-year studies in rats (0, 25, and 50 mg/kg) were based on the potentially life-threatening nature of the glandular stomach lesions and the decreased body weight gain compared to controls seen in the 62 and 125 mg/kg dose groups in the 13-week studies.

The final mean body weights of high-dose males and females were 91% and 89% of controls, respectively. The 2-year survival rates for males in the control, low-, and high-dose groups were 41/60, 30/60, and 24/60; survival rates for female rats were 37/60, 30/61, and 31/60.

Nonneoplastic and Neoplastic Effects in the 2-Year Studies: The principal toxic effects associated with the administration of titanocene dichloride for 2 years occurred in the stomach. The lesions in the stomach were seen at the 15-month interim evaluations and were similar to, but less severe than, those observed at 2 years. The lesions included focal erosions of the glandular mucosa with an associated inflammatory response, hyperplasia and metaplasia of the epithelium of the fundic glands, and fibrosis of the lamina propria and submucosa. Forestomach lesions included focal acanthosis (hyperplasia) and hyperkeratosis of the stratified squamous epithelium. Squamous cell papillomas of the forestomach were seen in four low-dose males, one high-dose male, one low-dose female, and two high-dose females; none were observed in controls. A squamous cell carcinoma of the forestomach occurred in one low-dose male and a benign basosquamous tumor occurred in one high-dose male.

Accumulations of macrophages with blue-gray pigment believed to contain titanium were present in many organs of dosed rats including the gastrointestinal tract, liver, lung, and lymph nodes. A dose-related increase in the incidence of inflammation of the nasal mucosa and lung also occurred and was attributed to reflux and/or regurgitation and aspiration of gavage solution due to the severe stomach lesions.

Genetic Toxicology: Titanocene dichloride was mutagenic in *Salmonella typhimurium* strain TA100 in the absence of exogenous metabolic activation (S9); it was not mutagenic in TA100 with S9, nor was it mutagenic in TA1535, TA1537, or TA98 with or without S9. Titanocene dichloride did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells, with or without S9.

Conclusions: Under the conditions of these 2-year gavage studies, there was *equivocal evidence of carcinogenic activity* of titanocene dichloride in male F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas, squamous cell carcinoma, and basosquamous tumor benign. There was *equivocal evidence of carcinogenic activity* of titanocene dichloride in female F344/N rats based on a marginal increase in the incidence of forestomach squamous cell papillomas.

Nonneoplastic lesions associated with the administration of titanocene dichloride for up to 2 years included erosions and inflammation of the gastric mucosa, hyperplasia and metaplasia of the fundic glands with fibrosis of the lamina propria in the glandular stomach, and acanthosis (hyperplasia) and hyperkeratosis of the forestomach epithelium.

Synonyms: Titanium ferrocene; biscyclopentadienyltitanium dichloride; dichlorodi-π-cyclopentadienyltitanium; dichlorobis(η^5-2,4-cyclopentadien-1-yl)titanium; dicyclopentadienyltitanium dichloride; dichlorodicyclopentadienyltitanium; dichlorotitanocene; dicyclopentadi-

enyldichlorotitanium; dichlorobis(π-cyclopentadienyl)-titanium; bis(η^5-cyclopentadienyl)titanium dichloride; dichlorobis(η^5-cyclopentadienyl)titanium; dichlorobis-cyclopentadienyl titanium; dichlorobis(1,3-cyclopentadiene)titanium; bis(cyclopentadienyl)dichlorotitanium

Report Date: September 1991

TR-400 Toxicology and Carcinogenesis Studies of 2,3-Dibromo-1-Propanol (CAS No. 96-13-9) in F344 Rats and B6C3F$_1$ Mice (Dermal Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-401 Toxicology and Carcinogenesis Studies of 2,4-Dirminophenol Dihydrochloride (CAS No. 137-09-7) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-402 Toxicology and Carcinogenesis Studies of Furan (CAS No. 110-00-9) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-403 Toxicology and Carcinogenesis Studies of Resorcinol (CAS No. 108-46-3) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-404 Toxicology and Carcinogenesis Studies of Diphenylhydantoin (Phenytoin) (CAS No. 57-41-0) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-405 Toxicology and Carcinogenesis Studies of C.I. Acid Red 114 (CAS No. 6459-94-5) in F344/N Rats (Drinking Water Studies)

C.I. Acid Red 114 is one of five chemicals being evaluated in 2-year carcinogenicity and toxicity studies as part of the NTP's Benzidine Dye Initiative. This Initiative was designed to evaluate representative benzidine congeners, benzidine congener-derived dyes, and benzidine-derived dyes. C.I. Acid Red 114 was nominated for study because of the potential for human exposure during production of bisazobiphenyl dyes and because benzidine, a structurally related chemical, is a known human carcinogen.

Toxicology and carcinogenesis studies were conducted by administering desalted, industrial grade C.I. Acid Red 114 in drinking water to groups of F344/N rats of each sex for 13 days, 13 weeks, 9 or 15 months, or 2 years. These studies were performed only in rats because studies of benzidine congeners were being performed in mice at the National Center for Toxicological Research (NCTR). Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary cells, and *Drosophila melanogaster*.

13-Day Studies: Rats were exposed to C.I. Acid Red 114 in drinking water at doses of 0, 10,000, 20,000, or 30,000 ppm. All control and dosed rats survived except one male rat in the 20,000 ppm dose group. Final mean body weights in the three dosed groups were 94%, 83%, or 77% of controls for males and 92%, 88%, or 80% of controls for females. Water consumption declined with increased dose. Clinical findings included red stained fur, ears, and tail in all test animals. On gross necropsy, organs and tissues were also stained red.

13-Week Studies: C.I. Acid Red 114 was administered in drinking water at doses of 0, 600, 1,200, 2,500, 5,000, or 10,000 ppm. All control and dosed animals survived until the end of the study. Final mean body weights in the five dosed groups were 97%, 89%, 87%, 87%, or 85% of controls for males and 97%, 94%, 94%, 92%, or 89% of controls for females. Water consumption was decreased in dosed animals. As was seen in the 13-day studies, major organs and tissues from treated animals were stained red. Kidney toxicity characterized by regeneration and karyomegaly of tubule epithelial cells with chronic inflammation was observed in female rats at doses of 1,200 ppm or above. Treatment-related increases in relative liver weights and elevated liver enzyme levels were seen in males and females, centrilobular pallor in the liver was seen in all male dose groups. Because of these body weight differences, decreases in water consumption, and organ toxicity, the doses chosen for the 2-year studies were 70, 150, and 300 ppm for males and 150, 300, and 600 for females.

2-Year Studies: Male rats received doses of 0, 70, 150, or 300 ppm of C.I. Acid Red 114, and female rats received 0, 150, 300, or 600 ppm. Seventy animals were in the control and high-dose groups, 45 in the low-dose groups,

and 75 in the mid-dose groups. Ten animals were evaluated from the control and high-dose groups at 9 months, and ten animals from all dose groups were evaluated at 15 months. The average amount of compound consumed per day was 4, 8, or 20 mg/kg for males and 9, 20, or 70 mg/kg for females.

Survival and Body Weights: Survival at 105 weeks for male rats receiving 0, 70, 150, or 300 ppm was 24/50, 15/35, 26/65, and 1/50; for females receiving 0, 150, or 300 ppm, survival was 36/50, 13/35, and 6/64. All female rats receiving 600 ppm died by week 89. The decreased survival in treated groups was due primarily to the development of chemical-related neoplasms. Of the surviving animals, the final mean body weights for males receiving 70 or 150 ppm were 94% and 90% of control and for females receiving 150 or 300 ppm, 99% and 84% of control. These weight differences began in the second year of the studies and were attributed in part to the development of neoplasms in the dosed groups.

Histopathologic Effects in the 2-Year Studies: At 9 and 15 months, a few neoplasms were seen in the liver, lung, clitoral gland, skin, Zymbal's gland, oral cavity epithelium, and small and large intestine, and the number of neoplasms at these sites increased as the studies progressed. At 2 years, there was a clear carcinogenic response in the skin, Zymbal's gland, and liver of male and female rats, and in the clitoral gland, oral cavity epithelium, small and large intestine, and lung in female rats. Treatment-related increases were also seen in the incidence in neoplasms of the oral cavity epithelium, adrenal gland, and lung of male rats, and in mononuclear cell leukemia and in neoplasms of the mammary gland and adrenal gland in female rats. The incidence of these neoplasms was generally lower, but was significant and considered to be marginally related to chemical treatment. The same neoplastic effects have been previously observed in some or all of the NTP studies with dimethoxybenzidine, dimethylbenzidine, or C.I. Direct Blue 15.

Genetic Toxicology: In a standard preincubation protocol, C.I. Acid Red 114 was mutagenic in *Salmonella typhimurium* strain TA98 in the presence of induced hamster liver S9, and an equivocal response was noted in strain TA100 with hamster liver S9. However, no significant mutagenic activity was noted in strains TA1535 or TA1537 with or without S9 activation. In a modified *S. typhimurium* gene mutation test which employed reductive metabolism followed by oxidative metabolism with S9 liver enzymes, C.I. Acid Red 114 was strongly mutagenic in strain TA1538. C.I. Acid Red 114 did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells with or without S9 activation; reductive metabolism was not used in these cytogenetic tests. No increase in sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* administered C.I. Acid Red 114 by feeding or injection.

Conclusions: Under the conditions of these 2-year drinking water studies, there was *clear evidence of carcinogenic activity* of C.I. Acid Red 114 for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, and liver. Increased incidences of neoplasms of the oral cavity epithelium, adrenal gland, and lung may have been related to chemical administration. There was *clear evidence of carcinogenic activity* for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity epithelium, small and large intestines, and lung. Increased incidences of mononuclear cell leukemia, mammary gland adenocarcinoma, and adrenal gland pheochromocytomas may have been related to chemical administration.

Synonyms: 1,3-Naphthalenedisulfonic acid, 8-((3,3'-dimethyl-4'-((4-(((4-methylphenyl)sulfonyl) oxy)phenyl) azo) (1,1'-biphenyl)-4-yl)azo)-7-hydroxy, disodium salt, Acid Leather Red BG, Acid Red 114, Amacid Milling Red PRS, Benzyl Fast Red BG, Benzyl Red BR, Cerven Kysela, C.I. 23635, Erionyl Red RS, Folan Red B, Kayanol Milling Red RS, Leather Fast Red B, Levanol Red GG, Midlon Red PRS, Milling Red B, Milling Red BB, Milling Red SWB, NCI C61096, Polar Red RS, Sandolan Red N-RS, Sella Fast Red RS, Sulphonol Fast Red R, Supranol Fast Red GG, Supranol Red PBX-CF, Supranol Red R, Telon Fast Red GG, Tertracid Milling Red B, Vondamol Fast Red RS

Report Date: December 1991

TR-406 Toxicology and Carcinogenesis Studies of γ-Butyrolactone (CAS No. 96-48-0) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)

γ-Butyrolactone is an intermediate in the synthesis of polymers used as film formers in hair sprays, blood plasma extenders, and clarifying agents in beer and wine. Toxicology and carcinogenesis studies were conducted by administering γ-butyrolactone (greater than 97% pure) in corn oil by gavage to groups of F344/N rats and B6C3F$_1$ mice of each sex, 5 days per week for 16 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Drosophila melanogaster*, and Chinese hamster ovary cells.

16-Day Studies: Groups of five rats of each sex received doses of 0, 75, 150, 300, 600, or 1,200 mg of γ-butyrolactone per kg of body weight and groups of five mice of each sex received doses of 0, 87, 175, 350, 700, or 1,400 mg/kg. All male and female rats given 1,200 mg/kg and one male rat given 600 mg/kg died within 3 days. The mean body weight gain of female rats given 600 mg/kg was significantly lower than that of the controls. Mean body weight gains of the other female dose groups and all male dose groups were similar to those of the controls. All of the male and four female mice receiving 1,400 mg/kg died during the studies. Mean body weight gains of dosed mice were generally similar to those of the controls. Rats

receiving 600 or 1,200 mg/kg and mice receiving 350 mg/kg or more became inactive or recumbent with irregular respiration following dosing.

13-Week Studies: Groups of 10 rats of each sex received doses of 0, 56, 112, 225, 450, or 900 mg of γ-butyrolactone per kg of body weight and groups of 10 mice of each sex received doses of 0, 65, 131, 262, 525, or 1,050 mg/kg. One female and all male rats given 900 mg/kg died during the studies. The final mean body weight and mean body weight gain of male rats receiving 450 mg/kg were significantly lower than those of the controls; final mean body weights and body weight gains of all female rat dose groups were similar to those of the controls. There was an increased incidence of focal inflammation of the nasal mucosa in rats administered γ-butyrolactone. Three male mice and one female receiving 1,050 mg/kg died from γ-butyrolactone toxicity during the studies. The mean body weight gain and final mean body weight of high-dose male mice were lower than those of the controls; the mean body weight gains and final mean body weights of dosed female mice were similar to those of the controls. No lesions related to the administration of γ-butyrolactone occurred in mice of either sex.

2-Year Studies: The doses administered to groups of 50 animals per sex were 0, 112, and 225 mg of γ-butyrolactone per kg of body weight for male rats; 0, 225, and 450 mg/kg for female rats; and 0, 262, and 525 mg/kg for male and female mice.

Body Weight and Survival in the 2-Year Studies: The mean body weights of male rats administered γ-butyrolactone were similar to those of the controls throughout the study. The mean body weight of high-dose females was lower than that of the controls after week 5 and was 10% to 20% lower than that of the controls throughout the second year. The survival of high-dose male rats was slightly higher than that of the controls (control, 24/50; low-dose, 27/50, high-dose, 32/50) due primarily to a lower incidence of mononuclear cell leukemia in the high-dose group (16/50, 15/50, 9/50). The survival of dosed females was similar to that of the controls (28/50, 27/50, 28/50).

The mean body weights of dosed male mice were lower than those of the controls throughout the study, but the differences in mean body weights decreased when male mice were housed individually at week 67. The final mean body weights of dosed male mice were 6% lower than that of the controls. Mean body weights of dosed female mice were also lower than those of the controls throughout the study, and the final mean body weights were from 14% to 17% lower than that of the controls. The survival in high-dose male mice was significantly lower than that of the controls (35/50, 30/50, 12/50) due to bite wounds and fighting in high-dose males recovering from the sedative effects of γ-butyrolactone. The survival of female dosed mice was similar to that of the controls (38/50, 34/50, 38/50).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: No increased incidences of neoplasms or nonneoplastic lesions in male rats were related to the administration of γ-butyrolactone for 2 years. In female rats, negative trends were observed in the incidences of cysts (42/50, 35/50, 23/50) and fibroadenomas of the mammary gland (22/50, 14/50, 6/50) and in cysts of the pituitary pars distalis (25/49, 13/37, 11/48). These decreases were considered to be related to γ-butyrolactone administration.

Increased incidences of proliferative lesions, primarily hyperplasia, of the adrenal medulla in low-dose male mice were associated with γ-butyrolactone administration (pheochromocytoma, benign or malignant: 2/48, 6/50, 1/50; hyperplasia: 2/48, 9/50, 4/50). The incidence of hepatocellular neoplasms in both dose groups of male mice was lower than the incidence in the controls (hepatocellular adenoma or carcinoma: 24/50, 8/50, 9/50).

Genetic Toxicology: γ-Butyrolactone was not mutagenic, with or without exogenous metabolic activation (S9), in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, nor did it induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered in feed or by injection. Positive results were obtained, however, in cytogenetic tests with Chinese hamster ovary cells; γ-butyrolactone induced sister chromatid exchanges and chromosomal aberrations in trials conducted in the presence of S9 activation.

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of γ-butyrolactone in male F344/N rats given 112 or 225 mg/kg or in female F344/N rats given 225 or 450 mg/kg in corn oil. There was *equivocal evidence of carcinogenic activity* of γ-butyrolactone in male $B6C3F_1$ mice based on marginally increased incidences of adrenal medulla pheochromocytomas and hyperplasia in the low-dose group. The sensitivity of the study in male mice to detect a carcinogenic effect was reduced by the low survival of the high-dose group associated with fighting. There was *no evidence of carcinogenic activity* of γ-butyrolactone in female $B6C3F_1$ mice given 262 or 525 mg/kg in corn oil.

A decreased incidence of hepatocellular neoplasms in dosed male mice and decreased incidences of mammary gland fibroadenomas and cysts and pituitary cysts in female rats were associated with the administration of γ-butyrolactone.

Synonyms: Dihydro-2(3H)-furanone (8CI) (9CI), 1,2-butanolide, butyrolactone, 1,4-butanolide, 4-butyrolactone, 4-hydroxybutanoic acid lactone, γ-hydroxybutyric acid cyclic ester, γ-hydroxybutyric acid lactone, γ-lactone 4-hydroxy-butanoic acid, butyric acid lactone, butyryl lactone, 4-hydroxybutyric acid lactone, tetrahydro-2-furanone, 4-butanolide, 4-deoxytetronic acid, γ-hydroxybutyrolactone

Report Date: March 1992

TR-407 Toxicology and Carcinogenesis Studies of C.I. Pigment Red 3 (CAS No. 2425-85-6) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

C.I. Pigment Red 3, a yellowish red solid, is widely used for coloring paints, inks, plastics, and rubber, and in textile printing. It is used in a wide range of consumer items such as wallpaper, typewriter ribbons, carbon paper, and art materials. Toxicology and carcinogenicity studies were conducted by feeding groups of F344/N rats and B6C3F$_1$ mice of each sex diets containing C.I. Pigment Red 3 (97% pure) for 2 weeks, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

2-Week Studies: Groups of five rats and five mice of each sex were given feed containing 0, 6,000, 12,500, 25,000, 50,000, or 100,000 ppm C.I. Pigment Red 3 for 2 weeks. No chemical-related deaths occurred in rats or mice. Final mean body weights of exposed rats and male mice were lower than controls; female mice that received 6,000 and 50,000 ppm had significantly increased final mean body weights compared to that of the controls. The feed consumption of treated rats and mice was slightly greater than that of the controls, suggesting that C.I. Pigment Red 3 had no adverse effects on the feed palatability. Dose-related decreases in erythrocyte counts and hematocrit values and an increase in reticulocyte counts were observed in rats. Changes in these parameters were observed in mice, but there were no clear, dose-related trends.

13-Week Studies: Groups of ten rats and ten mice of each sex were given feed containing 0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm C.I. Pigment Red 3 for 13 weeks. No chemical-related deaths were observed in rats or mice. The final mean body weights of exposed female rats were significantly lower than that of the controls; the final mean body weights of exposed male rats and exposed mice were similar to controls. There were significant increases in relative liver and kidney weights of exposed male rats. Increases in the relative liver weights in mice did not occur with a dose-related trend and thus they were not considered related to chemical administration. Sites for the toxicity of C.I. Pigment Red 3 were the bone marrow, kidney, liver, and spleen in rats. Lesions observed in rats included bone marrow hyperplasia, congestion and hematopoietic cell proliferation of the spleen, and iron-positive pigmentation of the spleen, kidney, and liver. Sites for the toxicity of C.I. Pigment Red 3 in mice were the liver, kidney, and spleen in males and the liver and spleen in females. Lesions noted among mice in the spleen were hematopoietic cell proliferation and iron-positive pigmentation. In the liver, there was hematopoietic cell proliferation in male and female mice. Cytomegaly occurred in the renal tubule epithelium of the male mouse kidney.

2-Year Studies: Doses selected for the 2-year feed studies were 0, 6,000, 12,500, and 25,000 ppm for rats and 0, 12,500, 25,000, and 50,000 ppm for mice. The dose selection for rats was based on body weight changes observed for females that received 50,000 ppm; the dose selection for mice was based on the lack of body weight depression or death at the doses tested during the 13-week studies. Concentrations higher than 50,000 ppm in the feed were not used because higher levels might have adversely affected the nutritional value of the diet during the 2-year studies.

Body Weight, Feed Consumption, Clinical Findings, and Survival in the 2-Year Studies: Final mean body weights for male rats that received 25,000 ppm, female rats that received 12,500 and 25,000 ppm, and male and female mice that received 50,000 ppm were more than 10% lower than those of the controls. Feed consumption of exposed rats and mice was similar to that of the controls. No clinical findings indicative of toxicity were observed in rats or mice. The survival of low-dose male rats was greater than that of the controls (0 ppm, 28/50; 6,000 ppm, 40/50; 12,500 ppm, 28/50; 25,000 ppm, 20/50). Survival of exposed female rats and exposed male mice was similar to the controls; the survival of high-dose female mice was significantly decreased compared to that of the controls (39/50, 37/50, 31/50, 25/50). The reduced survival in this dose group may have been due to the increased incidence of ovarian abscesses.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: Benign adrenal pheochromocytomas were significantly increased in the 12,500 and 25,000 ppm groups of male rats compared to the controls (22/50, 29/50, 35/50, 34/50). However, malignant neoplasms were not increased in incidence (6/50, 7/50, 10/50, 4/50). The incidence of adrenal pheochromocytomas in dosed groups exceeded the range for NTP historical controls for feed studies (22%-48%), and the increased incidence of this neoplasm was attributed to C.I. Pigment Red 3 administration.

Squamous cell papillomas of the skin occurred with a positive trend in male rats (0/50, 4/50, 2/50, 6/50), and the incidence in the high-dose group was significantly greater than that of the controls. A poorly differentiated squamous cell carcinoma (diagnosed as carcinoma) was observed in a control male. The historical control rate for squamous cell papillomas in NTP feed studies is low (16/800 or 2%, range 0%-4%), and the higher incidence of this tumor in male rats may have been caused by the administration of C.I. Pigment Red 3.

Hepatocellular adenomas occurred with a positive trend in female rats, with a significantly greater incidence in the high-dose group than in the control group (0/50, 0/50, 1/50, 10/50). This neoplasm has occurred in only one historical control group in NTP feed studies (3/800, range 0%-6%), and the increase in hepatocellular adenomas in female rats was attributed to chemical administration.

Chemical-related nonneoplastic lesions observed in the livers of male and female rats included eosinophilic or mixed type foci of cellular alteration. Foci were often accompanied by angiectasis and cystic degeneration in males and by granulomas and cholesterol pigmentation

in females. Chronic nephropathy occurred with increased severity in exposed male and female rats. The lesions were more severe in males than in females. Other lesions considered secondary to renal disease included parathyroid gland hyperplasia, fibrous osteodystrophy of the bone, and mineralization of various organs (stomach, intestine, heart, and blood vessels). The increased incidence of hyperplasia of the transitional epithelium of the renal papilla observed in treated rats was considered to be part of the chronic nephropathy.

Zymbal's gland carcinoma incidences were marginally increased in the mid- and high-dose male rats (0/50, 0/50, 2/50, 3/50). The incidence in the high-dose group was outside the NTP historical control range (0%-4%), and the Zymbal's gland carcinomas may have been related to C.I. Pigment Red 3 administration.

Mononuclear cell leukemias, mammary gland fibroadenomas, and preputial gland/clitoral gland adenomas occurred at lower incidences in exposed male and female rats. The decrease in mononuclear cell leukemia was attributed to the direct effect of C.I. Pigment Red 3 or its metabolites on the mechanism responsible for inducing leukemias in aging rats, while the decreased incidence of mammary gland fibroadenomas might be attributed to decreased body weights in female rats. The cause of the decreased incidences of preputial and clitoral gland tumors is unknown.

Tubule adenomas of the renal cortex occurred at a significantly higher incidence in high-dose male mice than in controls (0 ppm, 0/50; 12,500 ppm, 0/50; 25,000 ppm, 0/50; 50,000 ppm, 6/50). Because this tumor occurred only in exposed males and was outside the range for NTP historical controls in feed studies (0%-2%), renal cortical tubule adenomas in male mice were considered to be related to the administration of C.I. Pigment Red 3.

Follicular cell adenoma of the thyroid gland occurred with a positive trend in male mice (0/50, 0/49, 1/50, 5/50). The incidence in the high-dose group was significantly greater than that in the controls. This chemical-related effect is supported by the increased incidence of follicular cell hyperplasia. Because the incidence of this tumor exceeded the range of the historical controls from NTP feed studies (0%-4%), the increase of follicular cell adenoma was attributed to chemical administration. Female mice receiving C.I. Pigment Red 3 had a significant increase in follicular cell hyperplasia but showed no increase in tumor incidence at this site.

Focal renal tubule hyperplasia and cystic hyperplasia occurred in exposed male mice but not in the controls. Cytomegaly (karyomegaly) of the renal tubule epithelium was seen in all treated male mice. The severity of the accompanying chronic nephropathy was increased in both male and female mice.

Genetic Toxicology: C.I. Pigment Red 3 was mutagenic in *Salmonella typhimurium* strains TA100 and TA98 in the presence of exogenous metabolic activation (S9); no increases in gene mutation were observed in strains TA1535 and TA1537, with or without S9. C.I. Pigment Red 3 did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in either the presence or the absence of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *some evidence of carcinogenic activity* of C.I. Pigment Red 3 in male F344/N rats as exhibited by increased incidences of benign pheochromocytomas of the adrenal gland. The marginal increase in the incidences of squamous cell papillomas of the skin and Zymbal's gland carcinomas may have been related to C.I. Pigment Red 3 administration. There was *some evidence of carcinogenic activity* of C.I. Pigment Red 3 in female F344/N rats as indicated by the increased incidence of hepatocellular adenomas. There was *some evidence of carcinogenic activity* of C.I. Pigment Red 3 in male B6C3F$_1$ mice as exhibited by the increased incidences of tubule adenomas of the renal cortex and follicular cell adenomas of the thyroid gland. There was *no evidence of carcinogenic activity* of C.I. Pigment Red 3 in female B6C3F$_1$ mice that received 12,500, 25,000, or 50,000 ppm.

The incidences of mononuclear cell leukemia and preputial gland tumors in male rats and mononuclear cell leukemia, mammary gland fibroadenoma, and clitoral gland tumors in female rats were lower in the exposed groups. The incidences of liver foci were markedly increased in exposed male and female rats. The severity of chronic nephropathy was increased in male rats and to a lesser extent in female rats given C.I. Pigment Red 3. An increase in the severity of nephropathy was observed in male and female mice; cytomegaly (karyomegaly) of renal tubule epithelium was observed in male mice. Thyroid follicular cell hyperplasia occurred with an increased incidence in male and female mice receiving C.I. Pigment Red 3.

Synonyms: 2-Naphthalenol, 1-((4-methyl-2-nitrophenyl)-azo)-; Calcotone Toluidine Red YP; Fast Red A; Pigment Scarlet R; Recolite Fast Red RBL; Sengale Light Red B

Report Date: March 1992

TR-408 Toxicology and Carcinogenesis Studies of Mercuric Chloride (CAS No. 7487-94-7) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-409 Toxicology and Carcinogenesis Studies of Quercetin (CAS No. 117-39-5) in F344 Rats (Feed Studies)

This Technical Report was not a final publication at the time this "Compendium" was prepared.

TR-410 Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in B6C3F$_1$ Mice (Inhalation Studies)

Naphthalene, a white, crystalline powder, is used as a moth repellent and in the manufacture of phthalic and anthranilic acids, naphthylamines, and synthetic resins. The 2-year studies were conducted by exposing groups of male and female B6C3F$_1$ mice to naphthalene (>99% pure) vapor for 6 hours daily, 5 days per week, for 104 weeks. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

2-Year Studies: Groups of male and female mice were exposed to atmospheres containing 0 (75 mice per group), 10 (75 mice per group), or 30 ppm (150 mice per group) naphthalene. Mice from each group were included for 14-day hematology evaluations (male: 0 ppm, 5 animals; 10 ppm, 4; 30 ppm, 10; female: 0 ppm, 4; 10 ppm, 5; 30 ppm, 10). Mean body weights of exposed mice were slightly lower than those of controls throughout the studies. Survival of male control mice was significantly less than that of exposed mice; the lower survival was the result of wound trauma and secondary infections related to fighting among the group-housed mice (0 ppm, 26/70, 37%; 10 ppm, 52/69, 75%; 30 ppm, 118/133, 89%). Survival of exposed female mice was similar to that of controls (59/69, 86%; 57/65, 88%; 102/135, 76%).

Neoplastic and Nonneoplastic Effects in the 2-Year Studies: No increase in tumor incidence related to naphthalene administration was observed in male mice. In females, the incidence of pulmonary alveolar/bronchiolar adenomas was significantly greater in the high-dose group than in the controls (5/69, 7%; 2/65, 3%; 28/135, 21%). One other high-dose female had an alveolar/bronchiolar carcinoma. The combined incidence of alveolar/ bronchiolar adenomas and carcinomas in the high-dose females was above those for control female B6C3F$_1$ mice from NTP feed, water, and inhalation studies (91/1,166, 7.8%, range 0%-16%). These lung tumors were attributed to naphthalene exposure.

Nonneoplastic lesions attributed to naphthalene exposure were observed in the nose and lungs of mice of both sexes. In the nose, naphthalene exposure was associated with an increase in the incidence and severity of chronic inflammation, metaplasia of the olfactory epithelium, and hyperplasia of respiratory epithelium. Chronic inflammation in the lung was associated with chemical exposure.

Genetic Toxicology: Naphthalene was negative for the induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with and without exogenous metabolic activation (S9). In cytogenetic tests with Chinese hamster ovary cells, naphthalene induced sister chromatid exchanges with and without S9 activation. Exposure to naphthalene induced a significant increase in chromosomal aberrations in Chinese hamster ovary cells in the presence of S9.

Conclusions: Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity* of naphthalene in male B6C3F$_1$ mice exposed to 10 or 30 ppm. There was *some evidence of carcinogenic activity* of naphthalene in female B6C3F$_1$ mice, based on increased incidences of pulmonary alveolar/ bronchiolar adenomas.

In both male and female mice, naphthalene caused increased incidences and severity of chronic inflammation, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium in the nose and chronic inflammation in the lungs.

Synonyms: Naphthalin, Naphthene, Tar Camphor

Report Date: April 1992

TR-411 Toxicology and Carcinogenesis Studies of C.I. Pigment Red 23 (CAS No. 6471-49-4) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-412 Toxicology and Carcinogenesis Studies of 4,4'-Diamino-2,2'-Stilbenedisulfonic Acid Disodium Salt (CAS No. 7336-20-1) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-413 Toxicology and Carcinogenesis Studies of Ethylene Glycol (CAS No. 107-21-1) in B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-414 Toxicology and Carcinogenesis Studies of Pentachloroanisole (CAS No. 1825-21-4) in F344 Rats and B6C3F$_1$ Mice (Feed Studies)

This Technical Report was not a final publication at the time this Compendium was prepared.

TR-415 Toxicology and Carcinogenesis Studies of Polysorbate 80 (CAS No. 9005-65-6) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies)

Polysorbate 80 is a nonionic surfactant used widely as an additive in foods, pharmaceutical preparations, and cosmetics as an emulsifier, dispersant, or stabilizer. Toxicity and carcinogenicity studies were conducted by administering polysorbate 80 (which met all compendial specifications) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*.

14-Day Studies: Groups of five rats and five mice of each sex received diets containing 0, 3,000, 6,000, 12,500, 25,000, or 50,000 ppm polysorbate 80. All animals survived to the end of the studies. The mean body weight change of male rats that received 50,000 ppm was significantly lower than that of the controls. The mean body weight changes in all other groups of dosed rats and in all groups of dosed mice were similar to those of the respective controls. No clinical findings or changes in absolute or relative organ weights in rats or mice were related to polysorbate 80 administration.

13-Week Studies: Groups of 10 rats and 10 mice of each sex received diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm polysorbate 80. All animals survived to the end of the studies. The final mean body weights of dosed rats and mice were similar to those of the controls. No clinical findings, changes in absolute or relative organ weights, or gross or microscopic lesions in rats or mice were related to polysorbate 80 administration.

2-Year Studies: Doses for the 2-year studies were selected based on the lack of observed compound-related effects at the dose levels used in the 13-week studies. Groups of 60 rats and 60 mice of each sex received diets containing 0, 25,000, or 50,000 ppm polysorbate 80 for up to 103 weeks.

15-Month Interim Evaluations: Interim evaluations were performed on 7 to 10 rats and mice from each dose group at 15 months. There were no significant changes in absolute or relative organ weights. Incidences of hyperplasia and inflammation of the forestomach were increased in female mice that received 50,000 ppm. No other chemical-related lesions occurred in rats or male mice evaluated at 15 months.

Body Weights, Clinical Findings, and Survival in the 2-Year Studies: The mean body weights in male and female rats and male mice administered polysorbate 80 were similar to those of the controls throughout the studies. The final mean body weight of female mice receiving 50,000 ppm was 11% lower than that of the controls. No clinical findings were associated with administration of polysorbate 80. The survival of dosed male rats was lower than that of the controls (0 ppm, 29/50; 25,000 ppm, 18/50; 50,000 ppm, 18/50); the survival of dosed female rats and male and female mice was similar to that of the respective controls (female rats: 23/50, 25/50, 25/50; male mice: 33/49, 34/50, 32/50; female mice: 30/50, 28/50, 26/50).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: The incidence of adrenal medulla pheochromocytoma was marginally increased in high-dose male rats (21/50, 19/50, 29/50). The incidence of hyperplasia of the adrenal medulla was increased in low-dose male rats but not in high-dose male rats (11/50, 22/50, 12/50).

No chemical-related increases in the incidences of neoplasms occurred in male or female mice. The incidences of squamous hyperplasia and inflammation of the forestomach were significantly increased in high-dose male and female mice; forestomach ulcers were significantly increased in high-dose females.

Genetic Toxicology: Polysorbate 80 was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with or without exogenous metabolic activation (S9).

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity* for polysorbate 80 in male F344/N rats based on an increased incidence of pheochromocytomas of the adrenal medulla. There was *no evidence of carcinogenic activity* for polysorbate 80 in female F344/N rats or in male or female B6C3F$_1$ mice given 25,000 or 50,000 ppm.

Administration of polysorbate 80 was associated with inflammation and squamous hyperplasia of the forestomach in male and female mice, and with ulcers of the forestomach in female mice.

Synonyms: Glycol; sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives; polyoxyethylene (20) sorbitan mono-oleate; sorethytan (20) mono-oleate; polyethylene oxide sorbitan mono-oleate

Trade names: Alkamuls PSMO-20; Armotan PMO-20; Capmul POE-O; Drewmulse POE-SMO; Emsorb 2722; Glycosperse O-20; Glycosperse O20 Veg; Glycosperse O20X; Hetsorb O20; Industrol O20S; Laxan ESO; Liposorb O-20; Lonzest SMO-20; Montanox 80; Nikkol TO-10;

Protasorb O-20; Sorbitan mono-oleate polyoxyethylene; Sorlate; Tween 80; Monitan; Olothorb; Sorbimacrogol Oleate 300; T-Maz 80

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